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)

DPG (2, 3-diphosphoglyceric acid) will be used as a representative therapeutic vaccine. Five 'DPG-responders' will be treated (once a month) with DPG (100 µg/kg of body weight i.v.) and analyzed as described in Table 1. The monthly intervals were chosen because the level of γδ T-cell activation induced by a single DPG injection decreases after about 30 days (Poccia *et al.* 1999, Molecular Medicine **5,** 471-476, 1999). From the blood collections, the residual viable PBMCs and the plasma samples will be stored frozen at -132°C. Five control animals will be placebo-treated (injections of saline). The therapeutic vaccinations will commence three weeks after the SIV_{mac} challenge (biological isolate 251; 40 $TCID_{50}$ i.v.). [The SIV challenge time will be the first day in Week 0 in Table 1.]

Table 1. DPG therapeutic vaccinations with clinical endpoint data

WEEKS	-3	-1	0	2	4	8	12	16	20	24	28	32	36	40	44	48
Monthly DPG treatments																
CBC	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•
Chem20	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•
Flow Cytometry	•	•	•	•	•		•		•		•		•		•	
ICA	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Plasma Viremia	•	•	•	•	•			•			•			•		•
Plasma Viral RNA	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Viral DNA in PBMC	•	•	•	•	•			•			•			•		•
p27 Antigenemia	•	•	•		•			•			•			•		•
SIV Serology	•	•	•		•			•			•			•		
Blood draw volume (ml)	12	12	12	10	12	10	10	12	10	10	12	10	10	12	10	12

The activation of Vγ9Vδ2 T cells will be measured in terms of their antiviral activities (capacity to reduce virus production *in vitro*) and their capacity to produce cytokines and antiviral β-chemokines. The SIV disease progression in SIV-challenged rhesus macaques will be assessed - the immunological parameters (numbers and functions of lymphoid cells) and the *in vivo* virus load will be monitored (ELISA and real time PCR techniques). Survival times will be recorded. The influence of therapeutic vaccinations on prognosis and survival in SIV-infected animals will be determined. Successful outcomes may result in novel therapeutic vaccination strategies for clinical testing in human AIDS. In subsequent years, drugs that have already been FDA-approved for treating bone demineralization diseases in *Homo sapiens*, and that we and others have identified as 'stimulatory for γδ T cells *in vitro*' will be used in identical set-ups. [Currently, we have sufficient information about the DPG γδ T cell-stimulatory activity *in vivo* to proceed with our therapeutic vaccination experiments. The dose required for the *in vivo* activation of rhesus γδ T cells by FDA-approved (for human use) drugs such as sodium pamidronate is being determined by our Italian collaborators. This information will be available for the next phase of our studies in Year 2 and Year 3 covered by this protocol.]

Additional information:

Animals selected for the studies are subjected to serologic evaluation to document each animal's status with respect to herpesvirus B, SIV, STLV-1 and SRV.

CBC. Complete blood counts are done using a Sysmex F-800 Microcellcounter that provides a printout of the values for WBC, RBC, Hgb, Hcrt, MCV, MCH, MCHC and platelet counts. The differential white blood cell count and reticulocyte counts are determined manually by microscopic examination of stained blood smears. CBC are performed by a commercial vendor (General Medical Laboratories, Madison, WI).

Blood chemistries. When required, the General Medical Laboratories performs stat determinations for creatinine, blood glucose, BUN, SGOT, SGPT and potassium. Also, complete blood chemistry evaluations are performed by the same laboratory (General Medical Laboratories, Madison, WI) which is a clinical laboratory and all data are fully validated. These evaluations include values for glucose, urea nitrogen, creatinine, BUN/creatinine ratio, sodium, potassium, chloride, CO_2, calcium, phosphorus, total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, alkaline phosphatase, creatine kinase, amylase, LDH, uric acid, triglycerides and cholesterol.

Monitoring of Experimental Animals. All study related records and data, both hard copy and electronic, are archived permanently at the facilities of the Wisconsin Regional Primate Center (WRPRC).

b) Do any animals undergo any type of restraint beyond normal housing methods? **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? **NO** If YES, discuss level of restriction, expected consequences, and justification for such restrictions

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.) **NO** If YES, indicated nonstandard husbandry required and justification for this practice.