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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)

The experimental macaques will receive intranasal inoculation with 10^7 TCID50 of selected influenza virus, 2 monkeys per virus. We expect this to be a 60-day study. This will determine the efficacy of future studies using influenza as a vector.

The four monkeys involved in the project will be divided into 2 groups, each receiving a different strain of influenza virus intranasally.

Experimental Group	# of Animals	Treatments
A1	2	A/Memphis/88 (H3N2) Influenza ($1x10^7$ pfu)
A2	2	A/Ann Arbor/6/60 (H2N2) Influenza ($1x10^7$ pfu)

The experiments will be conducted according to the following schedule:

Group (n=4)	Influenza dose		Immunization Schedule (days)	
	Strain 1	Strain 2	Nasal washes	Blood draw
A1	X		Days 2,4,6,8	Days –14,0,14,30
A2		X	Days 2,4,6,8	Days –14,0,14,30

Preparation of Influenza:
Strains:
A/Memphis/88 (H3N2) Influenza

A/Ann Arbor/6/60 (H2N2) Influenza

Influenza strains will be available from collaborator Yoshi Kawaoka. The criteria for choosing the virus strains are as follows:
1.) The virus must replicate in the respiratory tract and be cleared within reasonable time (expect 7-10 days) and stimulate a vigorous responses.

b) The virus cannot cause a lethal infection or be associated with high morbidity.

c) The virus should be representative of circulating human strains, specifically of the H3N2 subtype, to avoid introduction of influenza strains into the human population for which preexisting immunity is not present.

d) Because live influenza virus are not permitted as human vaccines, the cold adapted attenuated influenza stain maybe the most useful. (Cold Adapted (ca) A/Ann Arbor/6/60 (H2N2)

Intranasal Infection:

Intranasal infection with influenza has never been documented in rhesus macaques. A study by our group in 1998 details how the procedure was done in cotton-top tamarins. "Five cotton top tamarins were infected intranasally with influenza virus reassoret strain A/X-31 (Beare and Hall 1971). Infections were carried out under ketamine anesthesia using a 1cc syringe fitted with an infant feeding tube extension. Each animal received $1x10^7$ egg infectious doses 50% (EID_{50}) of A/X-31 in 0.1 ml phosphate buffered saline." (Evans et al., 1998).

We will do the same procedure using $1x10^7$ egg infectious doses 50% (EID_{50})

Nasal washes :

Nasal washes will be collected on days 2,4,6,8 of the infection to determine the titer of the virus.

Blood Draws
The amount of blood obtained from each of these draws will be well under the limit of the WRPRC blood volume calculations (Bodyweight of animal (kg) x 65 x .10= Maximal volume of blood to be drawn at one time (in mls). 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) 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 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.

Blood samples are needed to monitor the infection. Blood draws will be done at day 14 pre-inoculation, to confirm that the animals are negative for influenza antibodies, ruling out prior influenza exposure. Blood will be drawn on days 0, 14 and 30 post-infection to determine CTL (cytotoxic T cell), Th (helper T cell) and antibody response to the virus. For these studies, 2 ml of plasma is necessary to obtain antibody titers. Therefore a 4-5 ml blood draw at each of the timepoints indicated will be sufficient. This is well below the WRPRC maximums.

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.