DEVELOPMENT OF A SUB-HUMAN PRIMATE MODEL FOR DENGUE HEMORRHAGIC FEVER

Principal Investigators : Donald S. Burke
Srisakul Kliks
Ananda Nisalak
Markpol Tingpalapong
George S. Ward
Michael R. Elwell

OBJECTIVE : To develop a reproducible model of dengue hemorrhagic fever in sub-human primates.

BACKGROUND : Many of the fundamental problems concerning the pathogenesis of dengue hemorrhagic fever (DHF), such as the role of enhancing antibodies, the requirement for specific sequences of serotypes, the types of cells infected *in vivo*, and the nature and source of the mediators of increased vascular permeability, could be resolved if a reliable animal model of DHF were available. Attempts to develop such a model in the past by other workers have been uniformly unsuccessful. Our recent epidemiologic studies of DHF in Thai infants, taken together with supporting laboratory data, have suggested a critical role for antibody-dependent enhancement of virus growth in cells with receptors for the Fc portion of IgG molecules. We therefore sought to develop a model for infant DHF based on our knowledge of current epidemiologic and laboratory information.

MATERIALS AND METHODS : A total of six trials were conducted, with inter-trial variations of the species of primates used, the age of primates used, the tuberculin skin test status of the monkeys used, the source of antibody used as the *in vivo* enhancing agent, the dose of the enhancing antibody, and the dose of virus administered.

1. Primates : All trials were conducted in the AFRIMS Veterinary Medicine facility using captive primates. Subject animals were held in individual cages in an air-conditioned room for the duration of the study. Body hair was clipped from all animals just before the start of each trial, in order to better observe possible skin lesions, as well as to promote slightly lower body and skin temperatures that might promote virus growth. Ambient temperatures were held at 24 - 27^{o}C, and primate rectal temperature regularly measured 38 - 39^{o}C in pre-inoculated and in uninoculated control animals. When an un-weaned infant monkey was used, the infant and dam were caged together in the study room. Blood samples were obtained by physically restraining the animals while a femoral venapuncture was performed; general anesthesia was not used. All monkeys in this series that weighed less than 4.0 kg were colony-born in the AFRIMS facility; all those weighing more were captured and purchased.

2. Tuberculin skin test status : After the completion of trials I and II, an outbreak of tuberculosis occurred in the general colony. We were aware of three lines of evidence suggesting that animals recently infected with

tuberculosis might be better experimental subjects than non-tuberculous animals : (a) in his studies, Halstead observed (unpublished observations) a fatal dengue infection in a monkey only once, and at autopsy that animal was found to have previously unrecognized miliary tuberculosis, (b) studies of the pathology of DHF in humans regularly show profliferation and increased phagocytic activity of the reticuloendothelial system (changes not typically associated with acute viral infections), and (c) both the total number of RE cells, as well as the number of Fc receptors per cell can be shown to be increased during tuberculosis. Therefore the opportunity was taken to use animals recently infected with tuberculosis as experimental subjects. In trial III all the animals used were tuberculin positive, but negative for overt disease as determined by physical examination and x-ray examination. When one of the animals in this experiment died of an illness similar to DHF (see below), our suspicion was increased that the RE system might require activation for DHF to occur, and we continued to use tuberculin positive but clinically healthy animals in trials IV - VI. The animals in trial III were not treated with antituberculosis drugs until after completion of the experiment, while animals in trials IV - VI were treated with isoniazid and streptomycin throughout the study period. (The effect of INH and SM on dengue virus growth *in vitro* in the P388D1 mouse macrophage cell line were cursorilly examined, and found to be insignificant).

3. Challange virus : The virus strain used throughout these studies was the dengue type 2 strain D80-616. This strain was isolated from a fatal case of infant DHF and could be shown to be readily enhanced in *in vitro* experiments. 0.5 milliliters of low LLC-MK2 passage virus (pass 2 or 3) was inoculated intravenously through the saphenous system. In trials I and II a dose of 10-5 PFU, and in trials III - VI a dose of 10-4 PFU, were administered.

4. Enhancing antisera : Four different serum preparations, all of which were shown in *in vitro* experiments to enhance the growth of virus strain D80-616 in P388D1 cells, were administered to experimental animals during the series.

2734/80 : This serum was obtained from the mother of DHF case D80-616 at the time that the child was admitted to the hospital. Prototype Den 2 PRNT50 = 1:660; peak enhancing titer *in vitro* using virus PFU yield method = 1:10,000 (100 fold).

1799/82 : This serum was obtained from the same woman approximately 18 months later.

0653/82 : This serum was obtained from the mother of DHF case D80-214 approximately 2 years after that infant died of DHF. Prototype Den 2 PRNT50 = 1:1040; peak enhancing titer *in vitro* using virus PFU yield method = 1:10,000 (23 fold).

4g2MAF : This preparation was a mouse ascitic fluid prepared by inoculation of "hybridoma" cells into the peritoneal cavity of primed mice. The monoclonal antibodies secreted by this hybridoma react with a major flavivirus group epitope on the V3 surface glycoprotein of dengue viruses as well as all other flaviviruses, and have been shown to enhance the growth of dengue viruses in

P388D1 cells.

Supplies of all human sera used were exhausted. If additional quantities of serum # 1799/82 had been available, further studies would have been conducted with this serum.

5. Doses of enhancing antisera : Since the exact site of the critical target cells infected in DHF is unknown, we were initially unsure if the enhancing sera should be preferentially distributed only in the plasma or throughout the extracellular space. For all experiments, the plasma volume was estimated as 40 ml per kg of total body weight, and the extracellular fluid volume as 25% of the total body weight. In trials I and II, one animal received a dose of serum 15 minutes before virus challenge, with the serum dose calculated to be diluted to an optimal enhancing concentration when distributed over the plasma volume. All other experimental animals received a dose of serum 24 hours before virus challenge, with the serum dose calculated to distribute over the extracellular volume. In trials I and II an effort was made to produce exactly the optimal enhancing concentration *in vivo*. This approach failed (see below). We therefore reconsidered our strategy, based on the following observations : (a) the duration of the "at risk window" for the development of DHF in humans is quite narrow, only 2 to 3 months (i.e., 2-3 IgG half-lives), suggesting that DHF may not develop if the level of enhancing antibodies is more than four-fold too low or four-fold too high, and (b) in a series of preliminary *in vitro* experiments we observed that dilutions of serum that were in the neutralization range in our standard "virus yield" enhancement assay regularly produced an increase in the number of infected cells, suggesting that we might be grossly missing the ideal *in vivo* enhancing dilution using our standard assay. Accordingly, in trial III we attempted to "bracket" the optimal dilution. When this strategy apparently succeeded in producing DHF in one of the animals, we subsequently used the same dilution of serum in trials IV and V as had been used in the ill monkey in trial III. When a new source of enhancing serum (4G2 MAF) was used in trial VI, again an attempt was made to find the optimal *in vivo* enhancing dose by "bracketing".

6. Clinical observations and measurements : All experimental animals were examined daily by one of the veterinarian co-investigators, and a standard clinical report form was completed daily (the same AFRIMS form was used as is routinely used for the recording of clinical information on human DHF cases). Vital signs were recorded daily, including rectal temperatures. Considerable difficulty was experienced in the measurement of arm blood pressures despite the use of restraining devices and pediatric size sphygmomanometers, so only systolic pressures were determined by palpation. Prechallenge and post challenge blood samples were obtained. Blood was collected in heparin-rinsed syringes with fresh neddles from the femoral vein, and one aliquot was added to a tube with one drop of heparin for CBC determinations, and another to a clean tube for viremia and antibody determinations. The total volume sampled per day was 2.0 ml from infant monkeys and 5.0 ml from adult monkeys. Because day to day variations often occurred in the platelet count of even the control monkey (no enhancing serum) in trial I, in subsequent trials "laboratory control" animals were included (no enhancing serum, no challenge virus).

7. Viremia determinations : Plasma samples from all animals were stored at -70°C until testing. Samples were screened by inoculation of a 1:10 dilution into 7-10 Toxorynchites splendens mosquitoes. Head squash preparations of 5 mosquitoes per sample were examined by direct immunofluorescent staining, and any sample with one or more positive mosquitoes was subsequently titered to endpoint by inoculation of serial 10 fold dilutions into mosquitoes. Five head squash preparations were examined for each plasma dilution, and the viremia level calculated according to the Reed and Munch method. Results were expressed as the mosquito ID-50's per mosquito inoculum (approximately 0.85 microliters). Thus a viremia titer of 10-3 as listed in the results section below is equivalent to 10-6.2 mosquito ID-50's per milliliter.

8. Antibody determinations : The HAI and PRNT50 antibody responses of infected animals were performed according to standard AFRIMS procedures.

9. Specimen codes : All inoculations were made using coded syringes so that the examining veterinarians were blinded as to which serum or virus inoculations were administered to which animals. Also, all blood collections were made into letter-coded tubes, so that all clinical laboratory and virus laboratory workers were also blinded as to the identity of the monkey from which the sample was obtained. Codes were not broken until after the completion of the trial and associated laboratory work.

10. Summary of experimental protocols : See Table 1.

RESULTS :

1. Tabular summary of data : Daily measurements of rectal temperatures, packed cell volumes, leukocyte counts, platelet counts, hemaglutination inhibiting (HAI) antibodies, plaque reduction neutralizing (PRNT50) antibodies, and viremia are presented in Tables 2, 3, 4, 5, 6, 7, and 8, respectively.

2. Clinical disease resembling DHF : Overall, 24 monkeys received some combination of enhancing sera and virus challenge. Only in trial III was there a strong suggestion that enhancing antibodies were important in the pathogenesis of severe dengue : in this experiment both monkeys that received the highest doses of the enhancing serum 1799/82 developed profound and sustained thrombocytopenia, and one of these (DA-9) went on to die on day 8 after inoculation of severe and generalized hemorrhages. The clinical illness in this monkey began on day 6 when scattered petechia appeared on the legs, and blood was noted in the feces. Later that day, the rectal temperature fell to subnormal levels, and the animal was lethargic and did not eat. The systolic blood pressure, however, remained normal. At no time was there evidence of ascites or clinically detectable pleural effusion, and the hematocrit showed a steady decrease, with no evidence of hemoconcentration. The animal died on day 8. Gross and microscopic post-mortem examination revealed widespread subcutaneous hemorrhage, especially in the femoral region, and diffuse hemorrhages in the pericardium, the papillary muscle, the submucosa of the colon, and alveolar spaces of the lung. A single enlarged bronchial lymph node was found to contain granulomata with acid fast bacilli typical of *M. tuberculosis*. All organs, including the lung parenchyma, were free of tuberculosis. Direct immunofluorescent staining of frozen sections of autopsy

specimens (performed by Dr. J. Dalrymple and colleagues at USAMRIID) revealed infrequent weak to moderately staining cells in the liver, spleen, thymus, and lung. Inoculation of 20% homogenates of tissues obtained at autopsy revealed that the spleen (10-6.7 MID50/gm), liver (10-5.6 MID50/gm), mesenteric lymph node (10-5.6 MID50/gm), and bone marrow (10-5.4 MID50/gm) contained the greatest amount of virus, with lesser amounts detectable in the lung, thymus, and kidney. On the day of death, the plasma of monkey DA-9 contained 10-5.7 MID50/ml.

3. Peak viremia titers : See figure 1.

CONCLUSIONS :

1. The viremia of experimental monkeys is reproducible and predictable. It begins on day 1-2, lasts for 5-7 days, and at its peak reaches 10-6 - 10-8 MID50/ml.

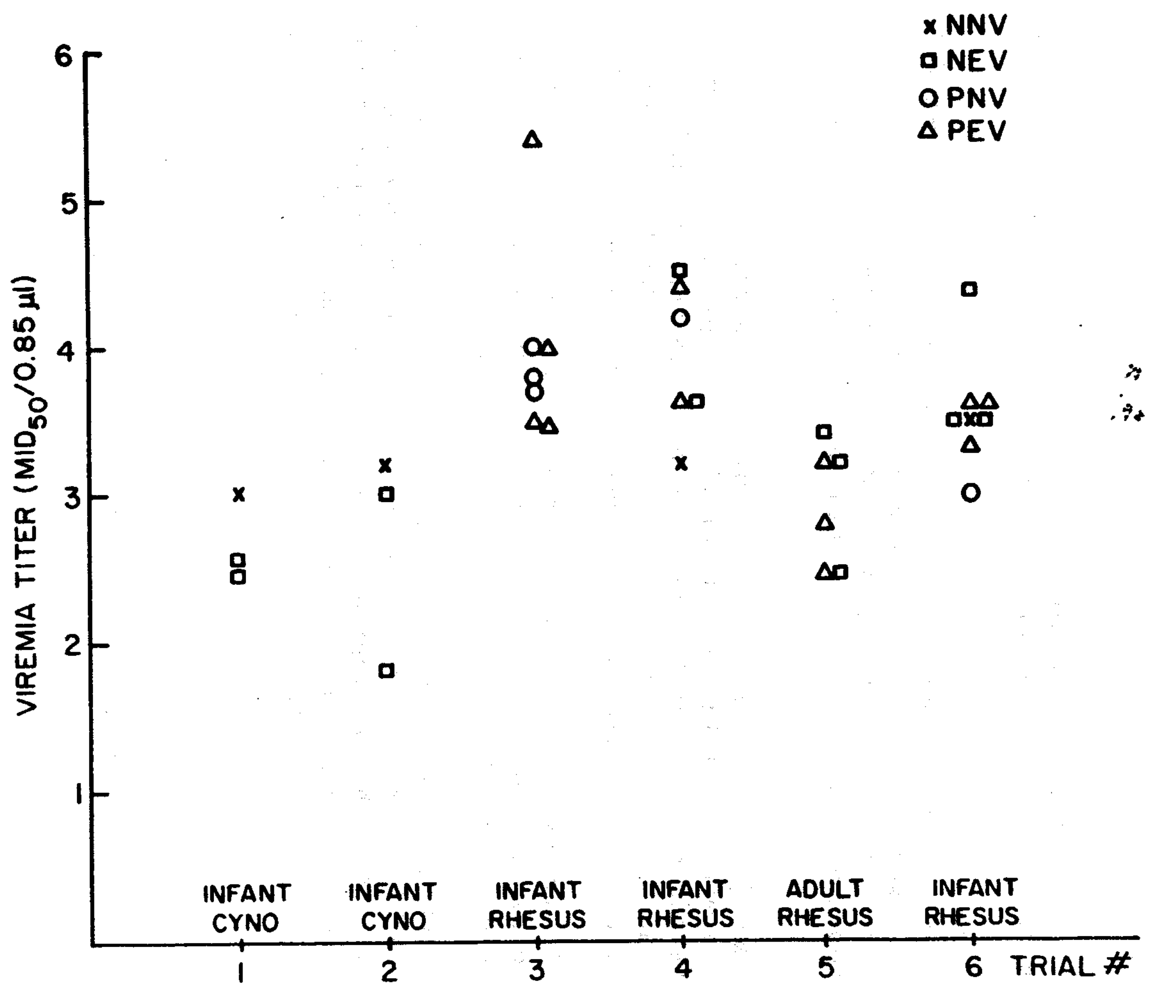
2. One of the 33 monkeys inoculated with the strain D80-616 in this series developed a fatal clinical disease which is can best be described as "dengue fever with hemorrhage", and another developed profound and sustained throbocytopenia. Thus, sub-human primates can develope severe disease due to dengue.

3. Based on this series of experiments it appears that the infant rhesus monkey is a more promising animal for the development of a DHF model than is either the adult rhesus or the infant cynomolgus monkey. This conclusion is based on the observations that viremias were reproducibly higher in the former than the latter, and that thrombocytopenia appeared only in the former.

4. In monkeys experiencing primary dengue, it appears that higher viremia levels are associated with severe disease (monkey DA-9 had the highest viremia of all monkeys examined).

5. Although the apparent success of the model in Trial III suggested that the combination of RE system activation and a precise enhancing antibody titer were required for the development of severe disease in infant rhesus, severe dengue illness could not be reproduced in subsequent trials following nearly identical protocols. Thus, the roles of both these factors remains unproven.

Figure I. PEAK VIREMIA TITER



Viremia (Log Mosquito ID-50 per 0.85 Microliters)

Trial	Monkey #	Treatment	Day 0	1	2	3	4	5	6	7	8	9-12	13-17
I	AF29	NNV	2.6	0	2.5	3.0	2.8	1.3	XXX	<1.0	XXX	0	0
	AF31	NEV	2.3	<1.0	2.6	1.0	1.5	0	XXX	0	XXX	0	0
	AF30	NEV	1.5	1.9	2.5	2.5	2.2	<1.0	XXX	0	XXX	0	0
II	AF35	NNN	0	0	0	0	0	0	0	0	0	0	0
	AF33	NNN	1.6	<1.0	3.0	3.2	2.6	2.2	XXX	<1.0	0	0	0
	AF32	NEV	2.2	1.3	3.0	2.2	1.6	<1.0	XXX	0	0	0	0
	AF34	NEV	1.8	1.5	1.8	1.8	1.8	<1.0	XXX	0	0	0	0
III	DA15	PNN	0	0	0	0	0	0	0	0	0	0	0
	DA18	PNV	<1.0	1.5	2.6	3.7	3.2	2.4	<1.0	<1.0	0	0	0
	DA12	PNV	<1.0	1.5	3.3	3.8	3.2	2.2	0	1.0	<1.2	0	0
	DA3	PNV	<1.0	<1.0	3.5	4.0	3.5	2.8	1.4	<1.0	<1.0	0	0
	DA27	PEV	0	<1.0	3.0	3.5	3.2	2.2	<1.0	0	0	0	0
	DA36	PEV	<1.0	1.4	3.5	3.5	2.6	1.8	0	0	0	0	0
	DA9	PEV	<1.0	<1.0	2.8	4.6	5.4	4.5	2.8	2.6	2.7	XXX	XXX
	DA4	PEV	0	<1.0	3.5	3.7	4.0	3.8	2.5	0	0	0	0
IV	DA10	PNN	0	0	0	0	0	0	0	0	0	0	0
	DA20	PNV	<1.0	0	1.6	3.8	3.5	4.2	2.5	1.0	0	0	0
	DA23	PEV	<1.0	<1.0	<1.0	3.4	3.5	3.6	3.2	1.8	0	0	0
	DA21	PEV	<1.0	<1.0	2.5	3.6	4.4	3.5	1.8	<1.0	0	0	0
	DA7	NNN	0	0	0	0	0	0	0	0	0	0	0
	DA11	NNV	<1.0	0	1.7	3.2	2.8	2.3	1.4	0	<1.0	0	0
	DA17	NEV	0	1.0	2.8	4.5	3.8	3.8	3.4	<1.0	0	0	0
	DA13	NEV	0	<1.0	2.5	3.6	3.2	2.5	<1.0	0	0	0	0
V	H34	PNN	0	XXX	0	XXX	0	XXX	0	XXX	0	0	0
	H178	PEV	0	XXX	3.2	XXX	2.8	XXX	0	XXX	0	0	0
	H172	PEV	<1.0	XXX	2.6	XXX	2.8	XXX	0	XXX	0	0	0
	H158	PEV	0	XXX	2.5	XXX	1.8	XXX	0	XXX	0	0	0
	H146	NNN	0	XXX	0	XXX	0	XXX	0	XXX	0	0	0
	H129	NEV	0	XXX	3.4	XXX	2.5	XXX	0	XXX	0	0	0
	H139	NEV	0	XXX	2.5	XXX	1.8	XXX	0	XXX	0	0	0
	H159	NEV	0	XXX	2.6	XXX	3.2	XXX	1.0	XXX	0	0	0
VI	DA10	PNV	0	2.2	3.0	2.3	1.2	<1.0	0	0	0	0	0
	DA15	PEV	0	1.3	2.5	3.3	2.7	1.3	0	0	0	0	0
	DA24	PEV	0	0	2.6	3.6	3.2	1.8	<1.0	<1.0	0	0	0
	DA25	PEV	1.3	<1.0	2.8	3.6	2.6	<1.0	<1.0	0	0	0	0
	DA1	NNN	0	0	0	0	0	0	0	0	0	0	0
	DA6	NNV	0	2.0	3.5	2.2	1.2	0	0	0	0	0	0
	DA29	NEV	<1.0	1.5	2.3	3.5	3.5	1.7	<1.0	0	0	0	0
	DA33	NEV	1.5	1.8	3.6	4.4	4.2	1.6	0	<1.0	0	0	0
	DA7	NEV	0	1.5	3.4	3.5	2.8	<1.0	<1.0	0	0	0	0

Morning Rectal Temperature (Degrees Centigrade)

Trial	Monkey #	Treatment	Day P1	P2	0	1	2	3	4	5	6	7	8	9-12	13-17
I	AF29	NNV	XXXXX	102.0	102.5	101.2	100.8	99.0	98.5	98.5	XXXXX	98.7	XXXXX	99.0	99.0
	AF31	NEV	XXXXX	101.5	101.6	100.7	99.5	99.7	100.2	100.5	XXXXX	101.5	XXXXX	101.0	100.5
	AF30	NEV	XXXXX	101.5	101.9	99.0	99.0	99.5	99.2	99.0	XXXXX	99.5	XXXXX	100.0	99.7
II	AF35	NNN	102.6	102.6	101.3	100.3	XXXXX	99.5	101.6	102.0	XXXXX	102.0	102.0	XXXXX	XXXXX
	AF33	NNV	100.8	100.5	101.6	98.9	XXXXX	98.8	97.4	98.4	XXXXX	97.2	96.0	XXXXX	XXXXX
	AF32	NEV	100.3	101.0	101.5	99.5	XXXXX	99.5	100.4	101.0	XXXXX	101.0	101.0	XXXXX	XXXXX
	AF34	NEV	101.5	100.7	101.0	100.0	XXXXX	100.5	101.8	102.9	XXXXX	101.6	102.0	XXXXX	XXXXX
III	DA15	PNN	XXXXX	XXXXX	XXXXX	101.6	101.6	101.8	101.6	102.2	101.8	102.4	100.8	101.4	XXXXX
	DA18	PNN	XXXXX	XXXXX	XXXXX	102.2	102.1	102.8	102.8	102.8	101.8	102.8	102.2	101.8	XXXXX
	DA12	PNV	XXXXX	XXXXX	XXXXX	101.2	101.8	101.8	101.2	101.6	100.0	101.0	100.6	100.6	XXXXX
	DA3	PNV	XXXXX	XXXXX	XXXXX	100.6	101.2	101.8	101.8	102.8	102.4	102.0	102.2	101.8	XXXXX
	DA27	PEV	XXXXX	XXXXX	XXXXX	101.4	101.6	101.2	100.8	102.7	102.0	102.4	102.2	102.0	XXXXX
	DA36	PEV	XXXXX	XXXXX	XXXXX	102.3	102.4	103.2	103.4	102.0	102.2	102.0	101.6	102.0	XXXXX
	DA9	PEV	XXXXX	XXXXX	XXXXX	100.4	101.2	102.0	101.8	100.4	98.8	94.2	XXXXX	XXXXX	XXXXX
	DA4	PEV	XXXXX	XXXXX	XXXXX	101.4	101.6	103.8	102.8	100.6	101.8	102.2	101.8	101.6	XXXXX
IV	DA10	PNN	102.0	102.4	102.6	101.4	101.2	101.5	XXXXX	103.0	101.6	102.3	102.5	102.8	103.0
	DA20	PNV	101.6	102.4	102.3	102.6	102.0	102.2	XXXXX	102.2	101.6	103.4	101.7	101.9	102.4
	DA23	PEV	100.4	100.7	100.8	100.6	101.5	101.8	XXXXX	103.6	101.3	102.6	101.6	101.4	101.3
	DA21	PEV	103.0	102.7	103.2	103.3	103.0	103.4	XXXXX	103.4	102.7	103.5	102.6	103.0	102.4
	DA7	NNN	101.7	102.2	103.2	103.5	102.7	102.2	XXXXX	103.4	102.7	102.6	102.6	103.0	102.3
	DA11	NNV	103.6	104.0	104.0	103.5	103.4	102.5	XXXXX	103.2	103.0	103.6	103.4	103.2	102.4
	DA17	NEV	102.6	103.0	102.6	102.6	102.8	103.0	XXXXX	103.0	103.0	102.7	102.5	102.4	102.4
	DA13	NEV	102.8	103.5	104.0	102.8	103.0	103.6	XXXXX	103.7	102.8	103.0	102.9	102.8	102.3
V	H34	PNN	99.0	100.5	101.2	XXXXX	101.2	XXXXX	100.6	XXXXX	100.3	XXXXX	100.4	99.4	101.1
	H178	PEV	98.6	100.8	101.6	XXXXX	101.6	XXXXX	101.2	XXXXX	99.0	XXXXX	100.6	99.7	100.9
	H172	PEV	100.0	98.6	99.3	XXXXX	100.8	XXXXX	101.3	XXXXX	98.6	XXXXX	100.8	100.4	101.4
	H158	PEV	103.0	100.5	100.0	XXXXX	101.0	XXXXX	100.8	XXXXX	101.2	XXXXX	101.0	99.4	101.5
	H146	NNN	100.2	102.0	98.0	XXXXX	99.9	XXXXX	99.0	XXXXX	100.0	XXXXX	100.8	101.0	100.3
	H129	NEV	100.6	99.0	98.6	XXXXX	99.3	XXXXX	98.6	XXXXX	97.8	XXXXX	99.0	98.6	99.4
	H139	NEV	99.0	100.5	100.5	XXXXX	102.6	XXXXX	101.6	XXXXX	101.6	XXXXX	101.7	101.6	102.6
	H159	NEV	100.0	100.5	96.0	XXXXX	99.6	XXXXX	100.7	XXXXX	99.8	XXXXX	99.0	99.2	101.0
VI	DA10	PNV	103.8	103.7	102.6	103.7	103.3	101.5	102.6	102.5	102.7	102.4	102.6	102.5	103.0
	DA15	PEV	102.2	102.0	102.5	103.4	101.6	101.4	101.0	101.2	101.1	101.6	100.1	101.5	102.9
	DA24	PEV	102.2	101.3	102.0	100.6	101.6	99.0	101.4	101.8	101.5	100.6	100.4	101.7	102.7
	DA25	PEV	102.4	102.3	103.5	101.7	101.8	101.5	101.4	102.5	101.5	102.0	101.7	102.0	102.6
	DA1	NNN	103.1	102.5	103.3	103.0	102.7	102.3	101.9	102.4	102.0	101.3	103.0	102.3	103.0
	DA6	NNV	102.6	103.0	103.2	101.7	102.6	101.9	102.1	101.4	101.9	102.1	102.3	102.5	102.0
	DA29	NEV	102.7	103.4	103.1	102.3	102.7	101.3	102.0	101.5	101.9	101.5	101.8	101.7	102.4
	DA33	NEV	102.7	103.4	103.5	102.4	101.9	101.6	101.7	102.0	102.5	102.4	102.2	102.0	102.6
	DA7	NEV	103.0	103.1	103.1	102.4	102.0	102.6	101.6	101.6	102.3	102.4	102.2	101.9	102.4

Packed Red Cell Volume (%)

Trial	Monkey #	Treatment	Day													
			P1	P2	P3	0	1	2	3	4	5	6	7	8	9-12	13-17
I	AF29	NNV	XX	43	XX	42	39	40	39	39	38	XX	39	XX	38	40
	AF31	NEV	41	43	XX	41	39	37	38	38	36	XX	38	XX	37	39
	AF30	NEV	40	40	XX	41	39	39	38	37	34	XX	37	XX	35	41
II	AF35	NNN	42	43	42	37	41	42	40	38	38	XX	41	39	38	40
	AF33	NNV	40	38	39	38	34	34	32	31	31	XX	32	33	32	35
	AF32	NEV	42	39	39	37	37	40	36	33	35	XX	37	36	37	40
	AF34	NEV	43	38	38	36	37	35	35	34	31	XX	36	36	34	41
III	DA15	PNN	40	40	40	38	42	38	35	40	38	36	38	37	40	40
	DA18	PNV	46	42	43	39	39	38	35	35	38	35	38	37	42	43
	DA12	PNV	49	44	47	43	43	47	43	44	45	43	43	46	46	46
	DA3	PNV	47	46	47	46	46	46	44	43	45	43	44	41	42	44
	DA27	PEV	41	38	41	38	36	XX	38	38	39	37	37	36	41	40
	DA36	PEV	40	37	40	36	36	35	33	33	34	35	34	33	40	40
	DA9	PEV	43	40	43	42	41	40	38	38	38	33	19	15	XX	XX
	DA4	PEV	43	41	43	38	39	41	37	38	41	39	38	37	36	37
IV	DA10	PNN	46	45	42	45	41	41	41	41	40	40	38	40	41	35
	DA20	PNV	38	36	39	38	37	29	35	32	34	32	34	33	35	36
	DA23	PEV	45	42	45	41	42	41	41	35	38	37	38	37	37	41
	DA21	PEV	37	37	42	40	38	36	37	37	36	35	XX	33	35	34
	DA7	NNN	43	42	39	37	39	41	39	38	37	38	38	39	38	36
	DA11	NNV	44	42	39	40	41	41	40	39	41	39	41	40	38	39
	DA17	NEV	48	46	43	44	43	43	41	39	38	37	35	38	39	41
	DA13	NEV	46	43	41	42	44	40	39	39	39	39	38	36	38	39
V	H34	PNN	39	38	40	40	XX	37	XX	37	XX	41	XX	38	39	37
	H178	PEV	39	39	41	38	XX	38	XX	34	XX	36	XX	36	41	32
	H172	PEV	-	40	43	40	XX	41	XX	37	XX	41	XX	38	43	36
	H158	PEV	46	44	45	43	XX	43	XX	39	XX	41	XX	41	38	40
	H146	NNN	44	42	43	43	XX	42	XX	43	XX	40	XX	44	38	41
	H129	NEV	38	39	40	39	XX	41	XX	36	XX	40	XX	38	37	40
	H139	NEV	45	44	46	44	XX	43	XX	41	XX	42	XX	39	40	42
	H159	NEV	40	43	41	41	XX	42	XX	38	XX	38	XX	43	36	41
VI	DA10	PNV	43	41	40	40	39	38	36	39	37	36	38	38	35	40
	DA15	PEV	37	38	38	38	37	37	34	37	35	35	37	37	35	36
	DA24	PEV	42	45	43	42	41	41	XX	38	37	39	39	39	38	41
	DA25	PEV	41	43	41	41	42	39	34	34	36	38	38	36	36	38
	DA1	NNN	39	43	42	41	38	XX	35	38	40	38	38	38	38	40
	DA6	NNV	42	40	41	37	36	35	33	36	39	37	38	37	38	41
	DA29	NEV	50	49	47	43	37	41	39	40	40	39	40	34	35	40
	DA33	NEV	44	44	45	42	39	37	33	35	36	38	37	35	37	41
	DA7	NEV	34	36	36	37	37	36	32	34	33	34	35	34	34	36

Platelet Count (X10^3 per mm^3)

Trial	Monkey #	Treatment	Day P1			0	1	2	3	4	5	6	7	8	9-12	13-17
I	AF29	NNV	XXX	XXX	231	340	225	166	185	127	067	XXX	168	XXX	065	125
	AF31	NEV	XXX	185	223	211	157	055	059	088	049	XXX	066	XXX	176	162
	AF30	NEV	XXX	154	362	159	213	102	196	128	054	XXX	078	XXX	142	109
II	AF35	NNN	676	563	600	369	765	498	510	608	209	XXX	867	470	498	377
	AF33	NNV	476	290	478	524	400	424	456	442	176	XXX	380	220	117	319
	AF32	NEV	742	413	316	608	555	566	482	573	386	XXX	613	459	697	680
	AF34	NEV	694	319	424	499	400	298	363	408	478	XXX	137	462	202	316
III	DA15	PNN	228	338	617	122	220	541	449	211	233	191	547	225	301	391
	DA18	PNV	070	156	210	113	176	346	185	101	137	082	252	132	148	134
	DA12	PNV	248	216	388	106	214	472	185	143	177	213	203	230	292	133
	DA3	PNV	141	190	198	117	176	397	028	130	142	115	282	151	149	228
	DA27	PEV	129	182	196	110	203	XXX	199	135	221	117	316	231	134	098
	DA36	PEV	139	238	195	038	170	075	055	114	085	079	120	111	154	065
	DA9	PEV	156	272	076	041	152	398	060	029	030	020	035	057	XXX	XXX
	DA4	PEV	085	086	052	046	139	107	047	041	019	028	026	041	065	102
IV	DA10	PNN	276	076	129	263	358	133	252	157	207	067	204	216	218	103
	DA20	PNN	597	363	348	394	303	037	321	249	226	101	163	194	348	282
	DA23	PEV	251	180	047	102	167	083	109	104	103	063	274	170	254	186
	DA21	PEV	290	195	191	355	321	191	253	111	122	076	XXX	155	198	217
	DA7	NNN	205	163	096	082	177	135	096	089	161	153	080	149	211	234
	DA11	NNN	479	760	114	044	203	143	095	094	109	079	123	140	161	236
	DA17	NEV	346	124	039	210	268	161	172	051	099	062	102	134	145	214
	DA13	NEV	320	231	064	224	264	108	109	077	127	091	192	219	143	157
V	H34	PNN	204	239	170	315	XXX	243	XXX	284	XXX	270	XXX	246	407	263
	H178	PEV	302	342	312	344	XXX	342	XXX	337	XXX	374	XXX	403	396	307
	H172	PEV	XXX	140	235	335	XXX	246	XXX	342	XXX	355	XXX	266	338	265
	H158	PEV	327	183	184	318	XXX	306	XXX	394	XXX	396	XXX	427	XXX	276
	H146	NNN	331	202	244	345	XXX	299	XXX	403	XXX	413	XXX	417	316	288
	H129	NEV	307	245	207	309	XXX	316	XXX	396	XXX	335	XXX	361	334	316
	H139	NEV	117	248	210	380	XXX	311	XXX	424	XXX	453	XXX	449	225	312
	H159	NEV	295	258	278	289	XXX	365	XXX	266	XXX	382	XXX	416	364	348
VI	DA10	PNV	301	367	226	385	179	310	276	111	183	184	172	147	165	185
	DA15	PEV	419	217	255	307	319	246	500	112	104	210	334	132	238	195
	DA24	PEV	195	172	152	408	200	490	XXX	140	368	355	176	061	098	134
	DA25	PEV	183	126	116	334	746	230	195	091	041	172	166	092	307	167
	DA1	NNN	218	289	328	375	270	XXX	292	041	126	095	106	184	259	253
	DA6	NNV	180	246	249	349	276	210	260	153	211	239	412	301	628	356
	DA29	NEV	411	630	437	659	246	598	480	423	148	368	327	207	186	300
	DA33	NEV	105	149	121	258	249	235	139	194	124	100	196	134	175	120
	DA7	NEV	125	211	153	213	398	237	128	199	144	107	181	162	132	XXX

Leukocyte Count (X10^3 per mm^3)

Trial	Monkey #	Treatment	P1	P2	P3	Day 0	1	2	3	4	5	6	7	8	9–12	13–17
I	AF29	NNV	XXXX	XXXX	13.0	10.0	19.3	18.2	11.9	13.2	11.9	XXXX	18.1	XXXX	11.5	25.5
	AF31	NEV	XXXX	XXXX	09.1	16.4	06.3	04.7	04.9	08.5	05.5	XXXX	07.0	XXXX	05.7	12.9
	AF30	NEV	XXXX	XXXX	11.3	06.9	11.7	06.0	06.9	06.4	06.7	XXXX	07.3	XXXX	08.5	12.7
II	AF35	NNN	16.9	14.3	14.9	12.5	11.4	13.2	12.8	09.2	14.8	XXXX	14.1	12.5	18.6	16.6
	AF33	NNV	18.4	19.5	19.3	07.4	12.4	09.0	10.1	09.9	11.7	XXXX	11.6	09.9	10.1	10.6
	AF32	NEV	15.4	19.7	19.5	13.1	12.3	14.8	11.9	09.8	11.5	XXXX	12.9	16.5	21.7	15.0
	AF34	NEV	13.7	12.0	09.8	15.2	09.9	09.1	09.8	07.6	09.8	XXXX	07.4	08.0	08.3	10.4
III	DA15	PNN	13.3	08.1	14.3	08.2	09.9	10.0	13.4	14.9	16.5	11.1	11.5	08.8	12.0	09.9
	DA18	PNV	17.0	15.3	14.7	23.5	10.5	10.1	09.3	11.0	08.2	08.4	09.5	10.9	12.2	14.7
	DA12	PNV	19.7	12.3	18.5	14.5	18.9	14.0	10.8	15.6	15.7	14.2	11.7	11.6	19.8	12.4
	DA3	PNV	13.6	11.3	16.2	12.5	18.0	12.0	07.8	06.4	09.6	07.2	05.2	07.1	10.7	09.9
	DA27	PEV	13.5	10.7	10.3	06.6	11.2	XXXX	09.5	09.2	13.2	09.7	12.0	06.5	09.9	09.8
	DA36	PEV	14.3	08.9	07.6	07.4	07.9	07.6	04.5	06.6	10.0	07.5	09.4	12.3	09.9	09.8
	DA9	PEV	21.0	15.1	15.1	16.0	15.1	13.5	09.4	12.2	14.4	11.2	05.9	13.7	XXXX	XXXX
	DA4	PEV	12.8	13.8	11.8	08.1	10.0	09.9	07.0	06.2	07.0	05.5	05.8	08.3	09.7	07.9
IV	DA10	PNN	10.5	08.5	14.1	09.0	12.1	11.7	10.3	13.4	08.2	07.9	08.3	05.7	11.6	12.1
	DA20	PNV	74.0	10.1	18.5	09.0	11.5	05.7	09.9	07.0	09.1	08.3	10.7	11.4	14.5	09.1
	DA23	PEV	10.3	11.8	09.4	10.2	15.3	12.6	08.2	06.3	05.5	07.2	10.6	10.3	14.0	11.7
	DA21	PEV	06.6	11.1	11.0	08.8	08.4	08.3	04.1	05.2	05.0	05.6	XXXX	05.6	06.8	06.8
	DA7	NNN	23.4	17.0	18.5	16.2	14.9	15.5	17.7	17.0	10.8	11.0	17.5	13.8	14.6	12.3
	DA11	NNV	14.4	08.2	14.1	15.1	12.1	13.5	11.9	10.2	12.2	09.7	11.5	12.5	10.1	20.5
	DA17	NEV	13.5	12.6	17.1	12.6	08.4	12.0	08.1	10.0	09.9	06.9	08.8	13.1	12.7	10.5
	DA13	NEV	09.1	11.4	11.0	10.0	11.4	09.9	09.2	08.5	09.7	08.6	07.9	08.5	08.1	10.5
V	H34	PNN	11.7	14.8	08.6	06.0	XXXX	08.1	XXXX	08.1	XXXX	09.2	XXXX	08.1	04.7	07.7
	H178	PEV	08.4	07.5	08.3	05.6	XXXX	06.4	XXXX	04.1	XXXX	04.6	XXXX	03.4	12.5	05.0
	H172	PEV	XXXX	10.8	06.8	04.9	XXXX	06.9	XXXX	03.9	XXXX	05.7	XXXX	05.2	04.1	06.7
	H158	PEV	11.3	08.4	10.7	06.9	XXXX	09.9	XXXX	05.8	XXXX	06.7	XXXX	06.4	06.0	05.7
	H146	NNN	08.5	10.5	15.5	12.1	XXXX	12.0	XXXX	10.9	XXXX	08.9	XXXX	13.4	05.1	15.6
	H129	NEV	07.1	08.3	05.6	05.4	XXXX	06.7	XXXX	03.4	XXXX	03.3	XXXX	05.4	04.6	05.0
	H139	NEV	13.2	10.6	06.1	07.6	XXXX	08.5	XXXX	04.7	XXXX	03.1	XXXX	05.2	04.8	04.3
	H159	NEV	07.0	12.0	05.7	07.0	XXXX	08.4	XXXX	03.0	XXXX	03.1	XXXX	05.6	05.0	04.8
VI	DA10	PNV	06.7	06.0	05.9	06.5	06.6	02.8	03.6	05.8	03.5	03.9	05.0	02.5	04.5	05.4
	DA15	PEV	09.4	12.2	08.7	09.4	11.2	09.4	09.9	09.1	07.9	07.8	10.5	09.4	08.0	06.1
	DA24	PEV	10.2	11.3	11.5	06.8	10.3	08.4	XXXX	03.1	06.4	09.5	08.1	08.9	07.7	09.2
	DA25	PEV	07.6	08.9	07.0	13.5	19.5	07.0	05.4	04.2	04.1	05.2	07.3	06.8	07.6	06.6
	DA1	NNN	06.3	07.8	09.7	11.1	08.0	XXXX	06.9	04.5	07.0	08.9	10.0	05.7	05.1	04.2
	DA6	NNV	08.3	10.7	07.4	06.3	09.6	08.4	03.3	04.7	05.3	05.5	06.0	05.8	04.7	07.4
	DA29	NEV	13.6	16.9	13.5	23.3	08.5	19.0	08.7	11.1	17.5	11.7	12.9	24.3	19.0	19.4
	DA33	NEV	09.7	09.6	10.2	14.5	08.3	07.4	06.3	07.3	08.0	07.6	10.6	06.5	06.7	06.8
	DA7	NEV	16.7	12.9	17.0	17.5	10.9	11.5	11.6	09.2	16.3	11.0	11.1	08.4	10.8	06.6

Experimental Dengue Infections in Monkeys : Enhancing Antibodies and Virus Doses

		Test Animals				Iq Pretreatment					Virus Inoculation		
Trial	Starting Date	Species	AFRIMS #	Wt	TBC Test	Plasma	Dose	Route	Day	Target Dilution	Virus	PFU Dose	Route
I.	22 Mar 82	Cyno	AF29	1.25	Neg	None	-	-	-	-	D80-616	10^5	IV
			AF31	1.60	Neg	2734/80	13	IV	0	1:5000 PL	D80-616	10^5	IV
			AF30	1.50	Neg	2734/80	75	IV	1	1:5000 ECF	D80-616	10^5	IV
II.	28 Apr 82	Cyno	AF35	1.70	Neg	None	-	-	-	-	None	-	-
			AF33	1.45	Neg	None	-	-	-	-	D80-616	10^5	IV
			AF32	1.45	Neg	2734/80	12	IV	0	1:5000 PL	D80-616	10^5	IV
			AF34	1.25	Neg	2734/80	62	IV	1	1:5000 ECF	D80-616	10^5	IV
III.	20 May 82	Rhesus	DA-15	1.36	Pos	None	-	-	-	-	None	-	-
			DA-18	1.81	Pos	Pool NHS	220	IV	1	1:2000 ECF	D80-616	10^4	IV
			DA-12	2.05	Pos	Pool NHS	550	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-3	3.5	Pos	Pool NHS	1700	IV	1	1:500 ECF	D80-616	10^4	IV
			DA-27	1.78	Pos	1799/82	110	IV	1	1:4000 ECF	D80-616	10^4	IV
			DA-36	1.56	Pos	1799/82	200	IV	1	1:2000 ECF	D80-616	10^4	IV
			DA-9	2.5	Pos	1799/82	620	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-4	2.8	Pos	1799/82	1410	IV	1	1:500 ECF	D80-616	10^4	IV
IV.	12 Jun 82	Rhesus	DA-10	2.6	Pos	None	-	-	-	-	None	-	-
			DA-20	2.0	Pos	Pool NHS	500	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-23	2.0	Pos	1799/82	500	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-21	1.6	Pos	1799/82	400	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-7	2.7	Neg	None	-	-	-	-	None	-	-
			DA-11	2.3	Neg	Pool NHS	570	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-17	1.9	Neg	1799/82	470	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-13	1.8	Neg	1799/82	450	IV	1	1:1000 ECF	D80-616	10^4	IV
V.	28 Jul 82	Rhesus	H-34	7.5	Pos	None	-	-	-	-	None	-	-
			H-178	6.5	Pos	0653/82	1600	IV	1	1:1000 ECF	D80-616	10^4	IV
			H-172	6.1	Pos	0653/82	1500	IV	1	1:1000 ECF	D80-616	10^4	IV
			H-158	10.0	Pos	0653/82	2500	IV	1	1:1000 ECF	D80-616	10^4	IV
			H-146	11.5	Neg	None	-	-	-	-	None	-	-
			H-129	8.4	Neg	0653/82	2100	IV	1	1:1000 ECF	D80-616	10^4	IV
			H-139	10.5	Neg	0653/82	2600	IV	1	1:1000 ECF	D80-616	10^4	IV
			H-159	8.8	Neg	0653/82	2200	IV	1	1:1000 ECF	D80-616	10^4	IV
VI.	31 Aug 82	Rhesus	DA-10	3.0	Pos	N1MAF	750	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-15	2.2	Pos	4G2MAF	550	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-24	2.1	Pos	4G2MAF	52	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-25	2.1	Pos	4G2MAF	5	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-1	2.9	Neg	None	-	-	-	-	None	-	-
			DA-6	2.3	Neg	N1MAF	580	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-29	2.0	Neg	4G2MAF	500	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-33	1.3	Neg	4G2MAF	47	IV	1	1:1000 ECF	D80-616	10^4	IV
			DA-7	2.8	Neg	4G2MAF	7	IV	1	1:1000 ECF	D80-616	10^4	IV

DEN 2 HAI Titer (Reciprocal)

Trial	Monkey #	Treatment	Day: Pl	0	1	2	3	4	5	6	7	8	9–12	13–17
I	AF29	NNV	10	10	10	10	10	10	10	XX	10	XX	20	80
	AF31	NEV	10	10	10	10	10	10	10	XX	20	XX	40	80
	AF30	NEV	10	10	10	10	10	10	10	XX	10	XX	40	80
II	AF35	NNN	10	10	10	10	10	10	10	XX	10	10	10	10
	AF33	NNV	10	10	10	10	10	10	10	XX	10	10	20	40
	AF32	NEV	10	10	10	10	10	10	10	XX	20	20	80	80
	AF34	NEV	10	10	10	10	10	10	10	XX	10	XX	20	80
III	DA15	PNN	10	10	10	10	10	10	10	10	10	10	10	XX
	DA18	PNV	10	10	10	10	10	10	10	10	10	10	160	320
	DA12	PNV	10	10	10	10	10	10	10	10	10	20	160	320
	DA3	PNV	10	10	10	10	10	10	10	10	20	10	80	640
	DA27	PEV	10	10	10	10	10	10	10	10	10	10	40	160
	DA36	PEV	10	10	10	10	10	10	10	10	20	40	320	640
	DA9	PEV	10	10	10	10	10	10	10	10	10	10	XX	XX
	DA4	PEV	10	10	10	10	10	10	10	10	40	80	320	640
IV	DA10	PNN	10	10	10	10	10	10	10	10	10	10	10	10
	DA20	PNV	10	10	10	10	10	10	10	10	10	40	80	1280
	DA23	PEV	10	10	10	10	10	10	10	10	10	20	40	160
	DA21	PEV	10	10	10	10	10	10	10	10	10	10	20	160
	DA7	NNN	10	10	10	10	10	10	10	10	10	10	10	10
	DA11	NNV	10	10	10	10	10	10	10	10	10	10	10	80
	DA17	NEV	10	10	10	10	10	10	10	10	20	40	80	320
	DA13	NEV	10	10	10	10	10	10	10	10	10	10	40	640
V	H34	PNN	10	10	XX	10	XX	10	XX	10	XX	10	10	10
	H178	PEV	10	10	XX	10	XX	10	XX	10	XX	20	80	320
	H172	PEV	10	10	XX	10	XX	10	XX	10	XX	40	40	320
	H158	PEV	10	10	XX	10	XX	10	XX	10	XX	20	20	320
	H146	NNN	10	10	XX	10	XX	10	XX	10	XX	10	10	10
	H129	NEV	10	10	XX	10	XX	10	XX	10	XX	160	320	640
	H139	NEV	10	10	XX	10	XX	10	XX	10	XX	10	40	320
	H159	NEV	10	10	XX	10	XX	10	XX	10	XX	20	160	320
VI	DA10	PNV	10	20	10	10	160	10	20	10	160	320	160	640
	DA15	PEV	10	40	40	20	10	20	10	40	10	40	10	80
	DA24	PEV	10	20	10	20	20	80	10	10	20	20	20	640
	DA25	PEV	10	10	10	10	40	80	10	20	20	20	40	1280
	DA1	NNN	10	10	10	10	20	20	20	10	10	10	10	10
	DA6	NNV	10	10	10	10	10	20	20	40	160	80	40	160
	DA29	NEV	10	10	10	20	10	10	10	40	160	20	160	640
	DA33	NEV	10	80	10	20	10	10	40	80	10	40	20	640
	DA7	NEV	10	10	10	10	10	10	10	20	160	80	320	320

DEN 2 PRNT 50 (Reciprocal)

Trial	Monkey #	Treatment	Day: Pl	0	1	2	3	4	5	6	7	8	9–12	13–17
I	AF29	NNV	10	11	27	10	24	19	140	XX	138	XX	165	3700
	AF31	NEV	42	10	23	45	150	18	60	XX	92	XX	41	380
	AF30	NEV	10	27	49	10	66	19	90	XX	30	XX	200	2400
II	AF35	NNN	10	10	10	10	10	10	10	XX	10	10	10	10
	AF33	NNV	10	10	10	10	10	10	10	XX	10	12	35	300
	AF32	NEV	10	10	10	10	10	10	13	XX	19	55	760	500
	AF34	NEV	10	10	10	10	10	10	10	XX	16	22	70	160
III	DA15	PNN	10	10	10	20	15	10	10	10	10	30	20	10
	DA18	PNV	10	10	20	27	10	10	15	50	60	250	1500	1700
	DA12	PNV	10	10	10	20	10	10	30	55	150	300	1000	2000
	DA27	PEV	10	30	10	10	10	10	10	50	20	10	155	1000
	DA36	PEV	10	15	10	10	10	25	22	33	22	55	400	1280
	DA9	PEV	10	50	10	10	20	25	15	23	35	30	XXX	XXX
	DA4	PEV	10	15	30	25	15	20	35	45	100	150	1100	2560
IV	DA10	PNN	10	10	10	10	10	10	10	10	10	10	10	10
	DA20	PNV	10	10	10	10	10	10	20	34	45	680	1200	1600
	DA23	PEV	10	10	10	10	10	10	10	36	52	415	1900	1600
	DA21	PEV	10	10	10	10	10	10	10	10	21	540	430	900
	DA7	NNN	10	10	10	10	10	10	10	10	87	12	14	10
	DA11	NNV	10	10	10	10	22	24	10	10	20	90	112	1100
	DA17	NEV	10	10	10	10	10	11	20	37	32	85	410	3300
	DA13	NEV	10	13	10	10	10	18	22	14	12	270	640	1600
V	H34	PNN	10	10	XX	10	XX	10	XX	10	XX	10	10	10
	H178	PEV	10	10	XX	10	XX	10	XX	23	XX	10	10	130
	H172	PEV	10	10	XX	10	XX	10	XX	22	XX	14	52	310
	H158	PEV	10	10	XX	10	XX	10	XX	28	XX	79	235	840
	H146	NNN	10	10	XX	10	XX	10	XX	10	XX	10	10	10
	H129	NEV	10	10	XX	10	XX	10	XX	100	XX	640	640	900
	H139	NEV	10	10	XX	10	XX	10	XX	19	XX	75	320	1050
	H159	NEV	10	10	XX	10	XX	10	XX	15	XX	23	27	125