

Dengue-2 Candidate Vaccine Studies: Challenge
of Immunized Monkeys with Southeast
Asian Wild Type Viruses

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OBJECTIVE : To challenge monkeys immunized with Dengue-2 candidate vaccine with local wild type dengue strains.

BACKGROUND : Ten Indian Rhesus monkeys (*Macaca mulatta*) were immunized (17 Jan. 1977) with a candidate Dengue-2 live attenuated virus vaccine strain (PR-159 (S1) Lot. No. 1 June 1976). (See Annual Progress Report 1976-1977). None of the ten monkeys developed detectable viremia. Four of these monkeys developed hemagglutination inhibition antibodies and six developed neutralizing antibodies against dengue-2 virus (KS-2472, MK2-4) by 30 days following vaccine administration. Of the six that developed antibody, three had neutralizing titers of 1:40 while three were 1:20. Five monkeys were inoculated with the Dengue-2 vaccine parent strain (PR-159, GM-6). All of these monkeys had proven viremia for three or more days (from the 2nd to the 8th day) and developed hemagglutination inhibition and neutralizing antibody (GMT's 1:210 and 1:600 respectively) by the 30th day following inoculation. The purpose of this experiment was to determine if the immunized monkeys were protected against infection with wild type dengue strains present in Southeast Asia.

METHODS : The ten monkeys previously immunized with the candidate 2 vaccine strains were divided into three groups :

Group 1 : This group consisted of two monkeys without neutralizing antibody (E290, E293) and another two with neutralizing antibody (E231, E299) (Table 1). This group received a wild type dengue-2 strain (BM50-76, Mk₂-2) which had been isolated from a mosquito collected from the home of a patient with dengue hemorrhagic fever and passed twice in LLC-Mk₂ cells. On initial isolation both large and small plaques were seen. The virus was administered in a dose of 0.5 ml containing 1.1×10^5 PFU.

Group 2 : Two vaccine immunized monkeys (F15, E297) and two monkeys (F17, F66) immunized with parent strain of dengue-2 virus (PR-159, GM-6) were included in this group (Table 2). These monkeys received a wild type dengue-3 strain (CH-1337-74, Mk₂-10) with a titer of 2.3×10^4 PFU/0.5 ml. This strain was

isolated from the serum of a dengue hemorrhagic fever patient and passed 10 times in LLC-Mk₂ cells.

Group 3 : This group included 2 vaccine immunized monkeys without neutralizing antibody (E294, E298) and 2 vaccine immunized monkeys with low (E229) and (E301) neutralizing antibody titers (Table 3). These monkeys received a booster with 3.3×10^2 PFU of candidate vaccine PR-159 (S1) lot. 1.

All monkeys were inoculated subcutaneously with 0.5 ml of the appointed strain of virus. The monkeys were examined the day before inoculation and daily throughout the course of the experiments.

The following blood specimens were obtained on day 0 prior to the inoculation and on days 1-10, 15, and 30 following immunizations : EDTA blood for hematology including hemoglobin, hematocrit, white blood cell and platelet count; serum for serological tests and SGPT; and heparinized blood for virus isolation from plasma and blood leukocytes. Virus isolation was performed by standard direct and delayed plaque technique in LLC-Mk₂ and by the mosquito-inoculation technique.

RESULTS :

Wild type dengue-2 challenges of immunized monkeys : The first group of monkeys (E290, 231, and 299) that were previously immunized with candidate dengue-2 vaccine were challenged with wild type dengue-2 strain (BM50-76, Mk₂-2) 4 months following the initial immunization. Viremia occurred in the three out of four monkeys (Table 1) (those three in which the neutralizing antibody titer had fallen to < 1:10 by the time of challenge). Viremia began on the third day following inoculation and lasted for 2 days. The monkey (E299) with a neutralizing titer of 1:100 in the prechallenge blood did not experience viremia. All monkeys developed high titers of neutralizing, hemagglutination inhibiting, and complement fixing antibodies (Table 1) in response to the challenge.

Wild type dengue-3 challenges of immunized monkeys : All monkeys in the second group developed viremia following dengue-3 challenge, and all developed a response of HI, CF, and NT antibodies (Table 2).

Reimmunization with candidate dengue-2 vaccine : In the third group, following booster immunization with dengue-2 candidate vaccine, no viremia was detected, but all monkeys showed CF, HI, and N antibody responses to dengue-2 (Table 3).

Challenge with wild type dengue-2 following booster immunizations : Three monkeys which has received the booster immunization were challenged approximately 10 months later with 2×10^6 PFU of wild type dengue-2 BM50-76 (Table 4). No viremia was documented in any of the challenged monkeys and again all redeveloped high titers of CF, HI, and N antibodies.

Table 2. Viremia and Antibody Responses of Dengue-2 Immunized Monkeys Challenged with Dengue-3 Virus.

Monkey Number	Original Inoculum (PFU)	Neutralize Titer at Day 30	Challenge Inoculum (PFU)	Viremia Days		Days Post Challenge	Reciprocal Antibody Titer											
				Plasma	LK*		HI				CF				NT			
							D-1	D-2	D-3	D-4	D-1	D-2	D-3	D-4	D-1	D-2	D-3	D-4
E-15	Vaccing (3.3×10^2)	10	D-3 (2.3×10^4)	4,5	4	d-0 d-15 d-30	0 320 160	0 1280 160	0 1280 640	0 1280 640	0 ND ND	0 128 64	0 ND ND	0 ND ND	10 320 550	0 80 320	0 160 160	
E-297	Vaccing (3.3×10^2)	80	D-3 (2.3×10^4)	4,5	None	d-0 d-15 d-30	0 320 320	0 1280 640	0 2560 1280	0 2560 1280	0 ND ND	0 128 256	0 ND ND	0 ND ND	0 250 320	0 80 160	0 160 160	
F-17	D-2 Parent (1.3×10^5)	222	D-3 (2.3×10^4)	4,5	4	d-0 d-15 d-30	20 2560 1280	160 1280 1280	40 2560 2560	40 5120 2560	ND ND ND	32 256 128	ND ND ND	ND ND ND	300 340 190	0 160 320	0 160 160	
F-66	D-2 Parent (1.3×10^5)	680	D-3 (2.3×10^4)	4,5,6	None	d-0 d-15 d-30	160 5120 5120	160 5120 2560	160 5120 2560	320 5120 2560	ND ND ND	32 56 128	ND ND ND	ND ND ND	640 >640 >640	0 160 320	40 160 160	

* Leukocytes
** Not Done

Table 3. Viremia and Antibody Response of Rhesus Monkeys Following Booster Immunization at 126 Days After Primary Immunization.

Monkey Number	Original Inoculum (PFU)	Neutralize Titer at Day 30	Challenge Inoculum (PFU)	Viremia Days		Days Post Challenge	Reciprocal Antibody Titers																			
				Plasma	LK*		HI			CF			NT													
							D-1	D-2	D-3	D-1	D-2	D-3	D-1	D-2	D-3	D-1	D-2	D-3	D-4							
E-294	Vaccine (3.3x10 ²)	0	Vaccine (2.5x10 ³)	None	None	d-0 d-15 d-30	0	10	0	0	0	0	ND**	4	ND	ND	0	0	0	0	0	0	0	0	0	
E-298	Vaccine (3.3x10 ²)	0	Vaccine (2.5x10 ³)	None	None	d-0 d-15 d-30	0	10	0	0	0	0	ND	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
E-228	Vaccine (3.3x10 ²)	20	Vaccine (2.5x10 ³)	None	None	d-0 d-15 d-30	10	40	20	12	80	40	ND	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0	0
E-301	Vaccine (3.3x10 ²)	53	Vaccine (2.5x10 ³)	None	None	d-0 d-15 d-30	0	80	20	0	40	20	ND	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0	0

* Leukocytes
** Not Done

Table 4. Dengue-2 challenge of rhesus monkeys after booster immunization with candidate dengue-2 vaccine*

Monkey Number	Viremia	Reciprocal Antibody Titers vs Dengue-2											
		CF				HI				NT			
		Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
		0	15	30	0	15	30	0	15	30	0	15	30
E-294	None	4	256	512	0	>10240	1280	60	>640	>640	60	>640	>640
E-298	None	8	256	256	40	>10240	2560	80	>640	>640	80	>640	>640
E-301	None	16	512	256	0	1280	1280	80	>640	>640	80	>640	>640

* Inoculum per monkey was 2×10^6 PFU of wild type dengue virus, BM-50-76.