Protection Against St. Louis Encephalitis and West Nile Arboviruses by Previous Dengue Virus (Types 1–4) Infection[1,2] (35098)

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Experimental support was sought to confirm the epidemiological hypothesis, reviewed elsewhere (1–3), that previous infection with one or more types of dengue (Den) virus produced significant resistance to the clinical manifestations of Japanese B encephalitis (JBE) and probably other encephalitides or fevers caused by closely related Group B arboviruses.

For reasons reviewed earlier by Hammon and Sather (4), West Nile (WN) virus and the young adult Syrian hamster were selected as the challenge virus and the experimental host of choice. In brief, WN is very closely related to JBE and SLE; the animals from the colony of hamsters used were known to be quite uniformly highly susceptible to fatal clinical disease from this virus following inoculation by a peripheral route, the natural route of infection for an arbovirus; and no other mature laboratory animal is similarly susceptible to either JBE or SLE virus. Furthermore, considerable experience had been gained in this laboratory with this animal in closely related types of experiments and with a suitable strain of WN virus (4–7).

A limited number of presumed dengue immune cynomolgus monkeys were employed for challenge with SLE virus itself, recognizing that clinical disease would not ensue, but using development of viremia as a criterion of lack of immunity. This also was based on previous experience in this laboratory with a similar model system by Imam and Hammon (8).

Materials and Methods. The viruses employed, sources, and passage levels were as follows: Dengue type 1 (Den-1), Hawaiian strain, passage 23 in mice, received in 1964 from Sabin as passage 20; Den-2, New Guinea "C" strain, mouse passage 23 received similarly at passage 19 level in 1955; Den-3, H87 and Den-4, H241 strains, the standard prototypes isolated here (9, 10) used in mouse passage levels 19 and 24, respectively. SLE virus, strain P-15, from the 1961 Florida epidemic (11) was used at mouse passage level 17, and WN strain B956, obtained from the American Type Culture Collection (at mouse passage level 26) and used at passage level 31.

Hamsters (golden Syrian) were from the Lakeview Hamster Colony and employed, except when otherwise stated, when about 5 or 6 weeks of age.

Immunization of hamsters with Den virus was by either the intraperitoneal (ip) or the intracerebral (ic) route, employing an active 5% w/v suckling mouse brain suspension in 3.0 or 0.05-ml amount, respectively, as specified in each experiment. Each suspension used was titrated at the time in suckling mice by the ic route and determined to be of expected potency. A rare hamster died from

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TABLE I. Peripheral West Nile Virus Challenge of Dengue 2 Virus-Immunized Hamsters.

West Nile challenge dilutions[a]	Expt. 1			Expt. 2	
	Immunized			Immunized	
	ic[b]	ip[b]	Normal controls	ic	Normal controls
10^{-2}				0/5[c]	
10^{-3}				1/5	
10^{-4}				2/5	
10^{-5}	1/5	2/5		1/5	
10^{-6}	1/5	1/4	5/5	3/3	5/5
10^{-7}	3/5	4/5	5/5		5/5
10^{-8}	3/5	2/5	2/5		5/5
10^{-9}			2/3		0/5
10^{-10}			0/3		0/5
LD_{50}			8.3		8.5

[a] 0.1 ml, sc; 36 and 35 days after dengue inoculation.

[b] Route of immunizing injection.

[c] Dead/inoculated; 21 day observation period.

TABLE II. Peripheral West Nile Virus Challenge of Dengue 1 Virus-Immunized Hamsters.

West Nile challenge dilution[a]	Immunized ic route[b]	Normal controls
10^{-3}	3/4[c]	
10^{-4}	2/4	
10^{-5}	3/4	
10^{-6}	2/4	5/5
10^{-7}	1/3	5/5
10^{-8}	4/4	5/5
10^{-9}		1/5
10^{-10}		1/5
LD_{50}		8.8

[a] 0.1 ml, sc; 37 days after dengue inoculation.

[b] Route of immunizing injection.

[c] Dead/inoculated; 21 day observation period.

dengue infection as a result of the ic inoculation, but none from the ip route. Immunized and controls of the same age were challenged with 10-fold graded doses of WN virus, 0.1 ml, subcutaneously (sc). Each suspension was also titrated in weanling mice by the ic route as a further check on potency. Hamsters were observed daily for signs of illness or death for a 21-day period, but in several experiments were held longer. A number of the immunized animals became ill, some with paralysis, but if they survived 21 days they usually recovered completely.

Ten newly purchased cynomolgus monkeys

TABLE III. Peripheral West Nile Virus Challenge of Dengue 3 and Dengue 4 Virus-Immunized Hamsters.

West Nile challenge dilution[a]	Den-3 immunized		Den-4 immunized		Concurrent controls[b]		Subsequent controls	
	ic[b]	ip[b]	ic	ip	"Young"	"Old"	"Young" 10–11 weeks	"Older" 14–15 weeks
10^{-2}	1/5[c]	3/5	2/5	3/5	—	—	3/4	2/4
10^{-3}	3/5	2/5	0/5	1/5	—	—	4/4	4/4
10^{-4}	4/5	2/5	4/5	2/5	—	—	4/4	4/4
10^{-5}	1/5	3/5	2/5	3/5	—	3/4	2/4	2/4
10^{-6}	3/5	2/5	2/5	3/5	2/4	3/4	4/4	4/4
10^{-7}	3/5	2/5	5/5	2/4	4/4	4/4	3/4	4/4
10^{-8}	—	—	—	—	2/4	4/4	4/4	—
10^{-9}	—	—	—	—	2/4	2/4	0/4	—
10^{-10}	—	—	—	—	0/4	4/4	0/4	—
10^{-11}	—	—	—	—	—	2/4	—	—
Total 10^{-2}–10^{-7}	29/60=48%		30/60=50%		16/20=80%		20/24=83%	20/24=83%

[a] 0.1 ml, sc; 37 days after Den-3 or Den-4 inoculation.

[b] Route of immunizing injection: ic immunized hamsters were 5–6 weeks of age at time of immunization ("young"); ip immunized hamsters were 3–4 months of age ("old"). Concurrent controls were from same group of animals as those immunized.

[c] Dead/inoculated; 21 days observation period.

were found to have Den virus neutralizing and/or hemagglutination-inhibition (HI) antibodies, assumed to be due to a previous naturally acquired infection, since Rudnick (12) finds them frequently with, or acquiring specific dengue antibody in the jungles of Malaya. These were given 1.0 ml of a 10% w/v suspension of suckling mouse brain SLE virus by the sc route. The virus suspension titered 9.8 logs/0.03 ml ic in weanling mice. Tests for viremia were made daily on days 1–11 in suckling mice by the ic route of inoculation.

Sera from some hamsters and monkeys, at stated intervals after immunization or challenge, were subjected to HI or neutralization (N) tests. The HI test employed the method of Clarke and Casals (13) and the N test utilized the mouse ic, serial virus dilution method, each as described by Hammon and Sather (14).

Results. Hamsters challenged with WN virus. Hamsters inoculated 35 or 36 days before with Den-2 virus were the first to be challenged with WN virus. Both ic and ip routes of Den inoculation were employed. Results of two trials are recorded in Table I. More protection was shown against large challenge doses than against the smaller, but marked protection occurred regardless of the route of dengue infection.

In a similar experiment with hamsters inoculated ic with Den-1 virus 37 days before, there was irregular evidence of partial protection, but not nearly as marked as with Den-2 (Table II).

TABLE IV. Results of Serological Tests on Hamsters.[a]

Hamster status and day following inoculation when bled	Animal no.	NI Den-2[b]	Hemagglutination-inhibition					
			Dengue virus type				West Nile	SLE
			1	2	3	4		
Den-2 immunized	1	2.8	1	*2*	1	5	4	5
day 33	2	3.9	0	*1*	1	3	5	4
	3	3.1	0	*1*	0	2	4	2
	4	4.0	2	*4*	3	7	8	7
	5	3.2	—	—	—	—	—	—
	6	3.9	0	*2*	0	4	5	5
	7	3.6	1	*1*	1	4	5	4
	8	3.9	1	*2*	1	4	5	4
Den-3 immunized	9		1	2	*3*	5	6	5
day 60	10		0	0	*1*	2	4	3
Den-4 immunized	11		0	2	0	*2*	6	2
day 60	12		0	2	1	*3*	4	3
	13		1	3	2	*4*	6	5
WN inoculated	14		4	5	5	9+	*9+*	9+
controls	15		3	3	3	9+	*9+*	9+
day 21	16		2	3	3	7	*9+*	9+
	17		4	5	5	9	*9+*	9+
	18		3	3	4	8	*9+*	8
Den-2 immunized	19		5	*5*	6	9+	*9+*	9+
and WN challenged	20		4	*5*	5	8	*9+*	8
day 21 post-WN	21		6	*7*	9+	9+	*9+*	9+
	22		7	*7*	7	9+	*9+*	9+
Normal controls,	23		0	0	0	0	0	0
no virus	24		0	0	0	0	0	0

[a] Dilution tube number at end point (0 = <1:10, 1 = 1:10, 2 = 1:20, etc.).

[b] NI = log neutralization index; Den-2, 1751 strain.

Den-3 and Den-4 viruses were utilized at the same time, both given to groups of animals by each of the two routes. However, the hamsters immunized by the ip route were older by 2–3 months than those immunized by the ic route. Controls for each age were employed. For reasons unknown, the control hamsters at both ages gave an unusual, irregular titration response in the high dilution ranges used (Table III). The challenge virus was titrated in weanling mice at the same time and again later, with expected results, $10^{8.2}$ and $10^{8.4}$ LD_{50}/0.03 ml. As a subsequent check, frozen ampoules of the same WN virus suspension were titrated in two age groups of hamsters: 10–11 weeks of age (comparable to the "young" immunized hamsters) and 14–15 weeks ("older hamsters") including lower dilutions with the results as shown (Table III). Apparently age did not affect the susceptibliity of the hamster to peripheral WN virus challenge.

The cross-protection test results indicate that both Den-3 and Den-4 provided slight but not marked protection against WN virus challenge.

Limited numbers of hamsters immunized with Den-2, -3, or -4 were sacrificed and bled, some prior to and some following WN challenge, and tested for certain antibody responses. Serological test results and times of bleeding are shown in Table IV. Only Den-2 immunized hamsters were tested for N antibodies; they were shown to have developed high titers of such for Den-2 virus. In general, the HI antibody responses to the Den viruses following immunization were relatively poor and definitely not type specific, but group specific, since titers to WN and SLE were generally as high or higher than that to the homologous Den virus. Survivors of high dilutions of WN virus not previously inoculated with Den virus responded with high titered and again broadly reacting antibody responses, highest to the homologous WN virus. Twenty-one days after WN virus challenge of Den-2 immunized hamsters there was a broad boost in HI antibody levels to the Den viruses, as well as to WN and SLE viruses, and for several viruses antibody levels were so high the end points were not attained.

Monkeys challenged with SLE virus. The monkeys challenged sc with SLE virus had been previously tested for HI antibodies with the 4 Den antigens, and those of JBE, WN, and Murray Valley encephalitis (MVE) and for N antibodies to Den-2. All had some type of positive response, though some at equivocal levels.

As observed in Table V, 6 of the 10 monkeys had detectable viremia on the first day following the heavy SLE challenge. In 2 animals the viremia persisted for 4 days as expected in a susceptible animal. These 2 had minimal detectable antibody, one by HI at only a 1:10 level for Den-4 and Murray Valley encephalitis (MVE) and with no detectable N antibodies to Den-2, the other with HI to Den-2 and Den-3 and MVE ranging from 1:10 to 1:80 and N antibody to Den-2 with >1.3 $\log_{10}$ neutralization index (quantity insufficient to test with lower dilutions of virus). The 8 animals with Den-2 neutralization indices ranging from 3.2–4.2 logs were viremic only on day 1 (4 monkeys) or showed *no* viremia (4 monkeys). The viremia occurring on day 1 may represent residual circulating virus due to the massive inoculum, or to a minimal degree of virus

TABLE V. St. Louis Encephalitis (P-15) Virus Infection in Cynomolgus Monkeys with Naturally Acquired Group B (?Dengue) Antibody.

Monkey	Log Neut. index Den-2	Viremia
3	0.0[a]	Day 1, 2, 3, 4
1	>1.3[b]	Day 1, 2, 3, 4
12	4.2	Day 1
14	4.2	Day 1
15	4.0	Day 1
18	3.9	Day 1
8	4.2	None
9	3.3	None
11	4.0	None
16	3.2	None

[a] HI antibody to Den-4 and Murray Valley encephalitis (1:10).

[b] HI antibody to Den-2 and -3 and Murray Valley encephalitis (1:10–1:80).

TABLE VI. HI Antibody Responses to Several Group B Viruses of Cynomolgus Monkeys Challenged with SLE Virus.

Monkey no.	Days after	Dengue virus types 1	2	3	4	West Nile	St. Louis encephalitis
3	0	0[a]	0	0	1	—	—
	17	1	3	2	7	7	8
	37	0	1	1	4	6	6
1	0	0	3	1	—	—	—
	17	2	2	2	6	8	7
	37	0	1	1	4	6	5
12	0	2	3	2	5	—	—
	17	4	6	4	7	8	8
	37	—	—	—	—	—	—
14	0	5	5	3	5	—	—
	17	7	8	7	9+	9+	9+
	35	5	5	4	6	8	7
15	0	1	4	2	5	—	—
	17	5	6	5	9+	9+	9+
	35	3	4	4	6	8	7
18	0	—	—	—	—	—	—
	17	3	1	3	3	5	4
	35	3	1	3	2	4	3
8	0	6	8	6	8	—	—
	17	7	8	7	9+	9+	9+
	35	6	7	6	8	9+	9+
9	0	3	4	3	4	—	—
	17	5	6	5	8	9+	9+
	35	4	4	4	6	8	7
11	0	3	4	4	5	—	—
	17	5	6	6	8	9+	9+
	35	4	4	4	5	8	6
16	0	—	—	—	—	—	—
	17	4	5	5	6	8	7
	35	4	3	4	5	8	6

[a] Dilution tube number at end point (0 = <1:10, 1 = 1:10, 2 = 1:20, 3 = 1:40, etc.).

multiplication. However, it appears that circulating SLE virus does *not* persist in the presence of adequate levels of neutralizing antibody against Den-2 virus.

An HI antibody response was elicited in all monkeys to SLE virus as well as to the 4 Den antigens and WN antigen. Table VI presents these antibody response patterns on days 17 and 37, with animals presented in the same order as in Table V. It will be noted that monkeys 3 and 1 with equivocal original serologic responses and bona fide SLE viremia tended to have less of an anamnestic response to Den-2, as well as to types 1 and 3 than most of the others, supporting the concept that they had probably not had a previous Den-2 infection, but more probably an infection by some other Group B agent, possibly Den-4, Zika, or another present in the area of their origin.

Summary. Hamsters inoculated with Den-2 virus were shown to survive peripheral challenge with large amounts of WN virus. Dengue types 1, 3, and 4 gave less complete

protection than Den-2. Cynomolgus monkeys with probable naturally acquired Den-2 neutralizing antibody were shown to be protected from a persistent viremia following SLE virus inoculation. Serological tests on both hamsters and monkeys were of the expected pattern but did not lead to an understanding of the specific mechanism involved in the cross protection. These results support epidemiological observations of the absence of severe epidemics of JBE, SLE, WN, and other Group B encephalitides in areas with endemic dengue.

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