AD-A143 082 EVALUATION IN RHESUS MONKEYS OF A BIVALENT LIVE ATTENUATED DENGUE VACCINE CONTAINING TYPES 2 AND 4 VIRUSES(U) PUERTO RICO UNIV SAN JUAN E KRAISELBURD

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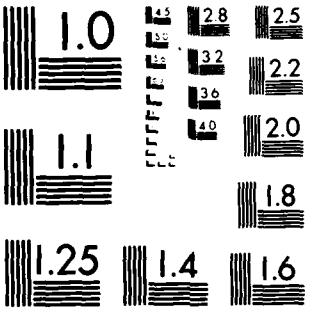

"Evaluation in Rhesus Monkeys of a Bivalent Live Attenuated Dengue Vaccine Containing Types 2 and and 4 Virusess"

Annual and Final Report

Edmundo Kraiselburd, Ph.D.

June 1984

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

DEN-2/S-1 Vaccine; DEN-4 Vaccine; Rhesus monkeys immunity.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of this study was to determine whether a bivalent (dengue-2-4) vaccine could confer immunity in rhesus monkeys against wild type virus infection →

Dengue antibody-free animals were s.c. inoculated with 0.5 ml of either DEN-2 (2.9×10^3 pfu/ml; 3.8×10^6 MID50/ML), DEN-4 (1.2×10^3 pfu/ml; 1.9×10^4 MID50/ml) or a 1:1 mixture of DEN-2 and 4 vaccines. Approximately six months after vaccination, animals were challenged with 10^4 MID50 (25 pfu) of wild type DEN-2 virus.

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20. Abstract (cont.)

It was found that:

1.) DEN-2/S-1 animals showed an anamnestic response as well as undetectable viremia after challenge with w.t. DEN-2 virus. Divalent vaccinees also showed an anamnestic response after DEN-2 virus challenge.

2.) DEN-4 vaccine did not elicit a measurable immune response in 6/8 animals as judged by PRNT, augmented N tests and by DEN-2 challenge experiments. An anamnestic response was observed in 2/8 animals.

3.) N antibody levels of most of the vaccinated animals declined markedly on or before post vaccination day 150.

4) Viral interference among the two different vaccine serotypes could not be demostrated.

Accomplishments: Dengue antibody-free animals were divided into four experimental groups. Group I (8 monkeys) were s.c. inoculated with 0.5 ml of a 1:1 diluted DEN-2/S-1 vaccine (2.9×10^3 pfu/ml; 3.8×10^6 MID_{50}/ml). Group II (8 monkeys) were inoculated with 0.5 ml of a 1:1 diluted DEN-4 vaccine (1.2×10^3 pfu /ml, $1{\cdot}9 \times 10^4$ MID_{50}/ml). Group III (13 monkeys) were inoculated with a 1:1 mixture of DEN-2 and DEN-4 vaccines. Group IV consisted of uninoculated (control) monkeys. Experimental animal were bled on post infection days 30, 60, 90, 120, 150, and 177 and N antibody titers against DEN-2 and 4 viruses were determined. Results from these experiments are shown in table 1. It can be seen that 6/8 DEN-2/S-1 vaccinated but only 2/8 DEN-4 vaccinated monkeys showed antibody response against the homologous wild type virus. 7/13 and 2/13 group III monkeys showed N antibodies against homologous wild type DEN-2 and DEN-4 virus, respectively. A X^2 test revealed that there was no statistically significant difference in seroconversion between monovalent and divalent vaccinees ($p<5\%$). This demonstrated that viral interference among the two different vaccine serotypes did not occur. In agreement with previous reports,(1) antibody levels of most of the vaccinated animals declined markedly on or before post vacination day 150.
PRNTs showed that the DEN-4 vaccine did not elicit a strong immune response in rhesus monkeys. This vas in agreement with results obtained in WRAIR which showed that only 2/5 human volunteers seroconverted after DEN-4 Vaccination (Dr. W. Brandt, personal communication).

Six months after vaccination, all animals were challenged with 10,000 MID_{50} (25 pfu; 1,000 monkey ID_{50}) of DEN-2 (PR159) virus (see Table II, page VI-8). Post challenge viremia was detected in 3/4 group 4 (control) monkeys, 1/13 group 3 (divalent vaccinees) and 5/8 group 2 (DEN-4 vaccinnees). No

Viremia was detected in group 1 (DEN-2 vaccinees).

As expected, all DEN-2 inoculated control animals showed a typical primary antibody response against DEN-2 virus. DEN-2/S-1 Vaccinees showed an anamnestic response against the challenge virus. This type of antibody response was also observed in 2/8 group 2 vaccinees and in all group 3 vaccinees. Therefore, although some animals failed to show a measurable primary antibody response after vaccination, challenge with w.t. DEN-2 showed that infection with vaccine did occur. We interpreted this data to mean that the standard PRNT used (2) was not the most sensitive assay to measure animal seroconversion. This hypothesis was tested in our laboratory using the "augmented" neutralizing antibody test. Using this test, we were able to show antibody response to homologous virus in sera taken from group 1 monkeys B851 and B853 and from group III monkeys B810, B817, B825, B806 and B804. Antibody titers ranged from 15 to 80 and were undetectable in some of the animals after post vaccination day 30. This test failed to reveal dengue 4 antibody titers in dengue-4 vaccinees which did not seroconvert, as measured by the standard PRN test.

In conclusion, these experiments showed that:

1. In agreement with previous work of Scot et al (1) DEN-2/S-1 vaccinated animals showed an anamnestic response as well as undetectable viremia after challenge with w.t. DEN-2 virus. Divalent vaccinees also showed an anamnestic response after DEN-2 virus challenge.
2. DEN-4 vaccine did not elicit a measurable immune response in 6/8 animals as judged by PRNT, augmented N tests and by DEN-2 challenge experiments. An anamnestic response was observed in 2/8 animals.
3. In agreement with previous reports, N antibody level of most of the vaccinated animals declined markedly on or before post vaccination day 150.
4. Viral interference among the two different vaccine serotypes could not be demostrated.

TABLE I: ANTIBODY RESPONSE OF RHESUS MONKEYS VACCINATED WITH WRAIR STRAINS OF DENGUE VIRUS.

Monkey Number	Vaccine Type	ANTIBODY TITERS ON POST-VACCINATION DAY 30	60	90	120	150	177
B840	D2	40	86	68	27	<10	<10
B851	D2	<10	<10	<10	<10	<10	<10
B873	D2	<10	72	14	<10	<10	<10
B814	D2	<10	14	84	74	80	18
B837	D2	74	11	17	33	26	37
B833	D2	40	160	80	150	159	320
B853	D2	<10	<10	<10	<10	<10	<10
B815	D2	<10	32	29	<10	<10	<10
B802	D4	<10	20	17	<10	<10	<10
B829	D4	<10	<10	<10	<10	<10	<10
B812	D4	<10	<10	<10	<10	<10	<10
B805	D4	<10	<10	<10	<10	<10	<10
B826	D4	<10	<10	<10	<10	<10	<10
B834	D4	<10	<10	<10	<10	<10	<10
B838	D4	52	27	<10	<10	<10	<10
B855	D4	<10	<10	<10	<10	<10	<10

		D2	D4	D2	D4	D2	D4	D2	D4	D2	D4	D2
B811	D2+D4	18	<10	30	<10	230	<10	110	<10	220	<10	96
B810	D2+D4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
B813	D2+D4	<10	<10	72	<10	310	<10	380	<10	270	<10	200
B822	D2+D4	115	<10	>160	<10	>160	<10	>160	<10	>160	<10	500
B827	D2+D4	58	<10	>160	12	64	>40	50	27	58	<10	130
B817	D2+D4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
B852	D2+D4	<10	<10	>640	>40	>640	29	>640	<10	590	<10	880
B835	D2+D4	11	<10	72	<10	120	<10	230	<10	125	<10	220
B825	D2+D4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
B845	D2+D4	<10	<10	135	<10	47	<10	15	<10	13	<10	10
B836	D2+D4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
B806	D2+D4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
B804	D2+D4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
B832	Control											
B831	Control											
B839	Control											
B286	Control	<10		<10		<10		<10		<10		<10
B311	Control											
B521	Control											
A962	Control											
A411	Control											

(All control monkeys)

TABLE II

Viremia and Antibody Response of Vaccinated Rhesus Monkeys After Challenge With Wild Type Dengue-2 Virus

Monkey Number	Vaccine Type	PRNT(Aug. Test)* Seroconversion		Antibody titers on Challenge day (177 days)		Day of viremia (pfu/ml)	D-2 N titers on post challenge days		
		D-2	D-4	D-2	D-4		10	30	60
B840	D2	+	N.D.	<10	N.D.	-	86	>640	800
B851	D2	-(+)	N.D.	<10	N.D.	-	380	540	660
B873	D2	+	N.D.	<10	N.D.	-	18	>640	2000
B814	D2	+	N.D.	18	N.D.	-	300	700	70
B837	D2	+	N.D.	37	N.D.	-	500	>640	960
B933	D2	+	N.D.	320	N.D.	-	490	>640	440
B853	D2	-(+)	N.D.	<10	N.D.	-	>640	>640	1400
B815	D2	+	N.D.	<10	N.D.	-	28	>640	N.D.*
B802	D4	N.D.	+	<10	N.D.	6(7.5),7(5)	>640	>640	3100
B829	D4	N.D.	-(-)	<10	N.D.	-	<10	250	900
B812	D4	N.D.	-(-)	<10	N.D.	5(2.5),7(2.5),8(7.5)	<10	>640	1250
B805	D4	N.D.	-(-)	<10	N.D.	-	<10	350	640
B826	D4	N.D.	-(-)	<10	N.D.	-	<10	>640	620
B834	D4	N.D.	-(-)	<10	N.D.	6(2.5)	<10	>320	>1280
B838	D4	N.D.	+	<10	N.D.	6(30),7(17.5)	225	>640	1050
B855	D4	N.D.	-(-)	<10	N.D.	7(10)	<10	640	580
B811	D2+D4	+	-	96	N.D.	-	3000	>640	>2560
B810	D2+D4	-(+)	-(-)	<10	N.D.	-	320	>640	>2560
B813	D2+D4	+	-	200	N.D.	-	190	135	150
B822	D2+D4	+	-	500	N.D.	-	2800	>640	1800
B827	D2+D4	+	+	130	N.D.	-	>1280	>640	640
B917	D2+D4	-(+)	-(+)	<10	N.D.	-	520	>640	>2560
B852	D2+D4	+	+	880	N.D.	-	2000	>640	1000
B835	D2+D4	+	-	220	N.D.	-	>5120	>640	600
B825	D2+D4	-(+)	-(+)	<10	N.D.	-	245	>640	280
B845	D2+D4	+	-	10	N.D.	-	>160	>640	2560
B836	D2+D4	-(+)	-(-)	<10	N.D.	5(2.5),6(7.5)	270	>640	2300
B806	D2+D4	-(+)	-(-)	<10	N.D.	-	620	>640	>2560
B804	D2+D4	-(+)	-(-)	<10	N.D.	-	>640	>640	>2560
B286	Control	-	-	<10	<10	8(2.5)	<10	1300	>2560
B311	Control	-	-	<10	<10	8(7.5)	<10	640	115
B521	Control	-	-	<10	<10	6(10),7(25),8(5),9(2.5)	<10	>640	>2560
B311	Control	-	-	<10	<10	-	<10	>640	>2560
B832	Control	-	-	<10	<10	- (Uninfected)	<10	<10	<10
B831	Control	-	-	<10	<10	- (Uninfected)	<10	<10	<10
B839	Control	-	-	<10	<10	- (Uninfected)	<10	<10	<10
A962	Control	-	-	<10	<10	- (Uninfected)	<10	<10	<10

*N.D.: This monkey died on post-challenge day 51 of reasons unrelated to dengue virus infection.

N.D.: Not done.

(+): *Indicates seroconversion to homologous virus as determined by the augmented neutralization test.

List of References

1. Scott, R McN, Nisalak, A., Eckels, K.H., Tingplapong, M., Harrison, V.H., Gould, D.J., Chapple, F.E., and Russell, P.K.: Dengue 2 vaccine, viremia and immune response in rhesus monkeys. Infect. Immun. 27, 181-186, 1980.

2. Russell, P.K., Nisalak, A., Sukhavachana, P., and Vivona, S.: A plaque reduction test for dengue virus neutralizing antibodies. J. Immunol. 99, 285-790, 1976.

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4. Asano, Y., Albrecht, P., Stagno, S. and Takahashi, M.: Potentration of neutralization of varicella-zoster virus by antibody to immunoglobulin. J. Infect. Dis. 146, 524-529, 1982.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978)

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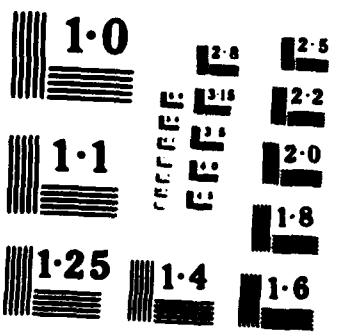
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SUPPLEMENTARY

INFORMATION

AD-A143082

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The purpose of this study was to determine whether a bivalent (dengue-2-4) vaccine could confer immunity in rhesus monkeys against wild type virus infection.

Dengue antibody-free animals were s.c. inoculated with 0.5 ml of either DEN-2 (2.2×10^3 pfu/ml; 3.8×10^4 MID_{50}/ml), DEN-4 (1.2×10^3 pfu/ml; 1.8×10^4 MID_{50}/ml) or a 1:1 mixture of DEN-2 and 4 vaccines. Approximately six months after vaccination, animals were challenged with 10^4 MID_{50} (25 pfu/kg) wild type DEN-2 virus.

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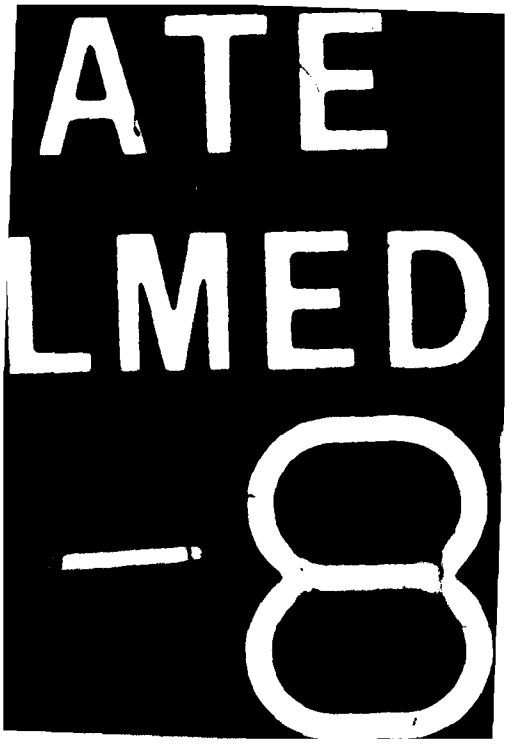
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