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COMPARATIVE INFECTIVITY DETERMINATION OF DENGUE VIRUS VACCINE
CANDIDATES IN RHESUS MONKEYS, MOSQUITOES, AND CELL CULTURE

ANNUAL REPORT

EDMUNDO KRAISELBURD

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University of Puerto Rico
G.P.O. Box 5067
San Juan, Puerto Rico 00936

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Vaccine Candidates in Rhesus Monkeys, Mosquitoes, and
Cell Culture

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Edmundo Kraiselburd

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DA335475

University of Puerto Rico
G.P.O. Box 5067
San Juan, Puerto Rico 00936

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Evaluation of Denque-2 vaccines:

I DEN-2 S16803 STRAIN

On September 4, 1990, sixteen rhesus monkeys were sent from the Caribbean Primate Center (Sábana Seca) to the Animal Resources Facilities at the Medical Sciences Campus. The monkeys were bled on September 4, and on September 17, and the sera were stored at -20°C. The Plaque Reduction Neutralization test (PRNT), and the hemagglutination inhibition (HI) tests were performed using these serum samples (Pre-vaccination bleds).

The PRNT was performed using LLC-MK2 cells (sent by Dr. Goro Kuno CDC, San Juan Laboratories) grown in multiwell plates as described before (see Second Semi-Annual Report, August 31, 1990).

Vaccines and the other materials were received on 10/4/90 from Dr. Kenneth Eckels (Walter Reed Army Institute of Research).

The following vaccines of dengue 2 were received: PDK-10, PDK-20, PDK-30 and PDK-50.

On October 15, 1990 groups of four monkeys were inoculated s.c. with 0.5 ml of each candidate vaccine. The titration of the four vaccine candidates were made on the same day of inoculation using LLC-MK2 cells.

Vaccine titers obtained were as follows:

<u>Vaccine</u>	<u>LLC-MK2</u> <u>(Pfu/ml)</u>
PDK 10	7.65 X 10 ⁵
PDK 20	4.35 X 10 ⁵
PDK 30	4.20 X 10 ⁵
PDK 50	1.16 X 10 ⁶

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As stated above, all animals were vaccinated with 0.5 ml of above vaccines, with the exception of PDK-50 (that was diluted 1:7 before inoculation, as instructed by Dr. Eckels). Diluent used was sent by WRAIR.

After vaccination, animals were bled for 10 consecutive days. As agreed, these samples were sent to WRAIR for viremia studies. On days 30 and 60 post vaccination (Nov. 14, and December 12, 1990) animals were bled and PRNTs against Dengue-2 (NGC strain) and Dengue-2 (S16803), were performed.

Results of these tests are presented in Tables I & II (enclosed).

TABLE I

ANTIBODY RESPONSE OF MONKEYS INOCULATED WITH DIFFERENT DEN-2
VACCINE CANDIDATES

Monkey No.	Vaccine	Vaccine Innoculum Pfu/.5ml	N. Antibody Titers*	
			day 30	day 60
	PDK-10	3.8×10^5		
R04			110	>640
R09			260	320
R17			>640	>640
R30			640	110
	PDK-20	2.18×10^5		
R02			580	>640
R19			600	460
R49			>640	>640
R54			>640	>640
	PDK-30	2.1×10^5		
R11			160	350
R18			<10	<10
R21			>640	300
R37			160	140
	PDK-50	0.83×10^5		
R05			32	140
R08			39	>640
R15			100	350
R36			>640	>640

*Neutralizing antibodies titers were obtained by the PRNT test, using the NGC strain of DEN-2.

TABLE II

ANTIBODY RESPONSE OF MONKEYS INOCULATED WITH DIFFERENT DEN-2
VACCINE CANDIDATES

Monkey No.	Vaccine	Vaccine Innoculum Pfu/.5ml	N. Antibody Titers*	
			day 30	day 60
	PDK-10	3.8×10^5		
R04			340	640
R09			560	400
R17			600	580
R30			500	500
	PDK-20	2.18×10^5		
R02			>640	700
R19			>640	>640
R49			>1280	>640
R54			>1280	>1280
	PDK-30	2.1×10^5		
R11			>640	160
R18			<10	<10
R21			>640	>640
R37			>640	460
	PDK-50	$.83 \times 10^5$		
R05			64	94
R08			155	>640
R15			160	82
R36			640	>640

*Virus used for PRNT: Parent S16803 STRAIN

II Dengue-2 S16681 STRAIN:

On February 12, 1991, sixteen rhesus monkeys were sent from the Caribbean Primate Center (Sábana Seca) to the Animal Resources Facilities at the Medical Sciences Campus. The monkeys were bled on February 12, and on February 26, and the sera were stored at -20°C . The Plaque Reduction Neutralization test (PRNT) and the hemagglutination inhibition (HI) test were performed using these serum samples (Pre vaccination bleeds).

The PRNT was performed using LLC-MK2 cells (sent by Dr. Goro Kuno CDC, San Juan Laboratories) grown in multiwell plates as described before.

Vaccines and the other materials were received on 4/4/91 from Dr. Kenneth Eckels (Walter Reed Institute of Research).

The following vaccines of dengue 2 (strain S16681) including the parent seed were received: PDK-11, PDK-40 and PDK 53.

On April 22, 1991 groups of four monkeys were inoculated s.c. with 0.5 ml of each candidate vaccine. The titration of the four vaccine candidates were made on the same day of inoculation using LLC-MK2 cells.

Vaccine titers obtained were as follows:

<u>Vaccine</u>	<u>LLC-MK2 (CDC)</u> (Pfu/ml)	Titers reported by WRAIR (Pfu/0.2 ml)
S16681	3.3×10^7	1.8×10^6
PDK 11	7.9×10^6	4×10^6
PDK 40	2.2×10^5	2.2×10^5
PDK 53	3.3×10^4	2.2×10^5

Shown on the right column are the titers obtained at WRAIR. These titers were different to those obtained in our laboratory, using LLC-MK2 cells received from the local CDC laboratory. Very small plaques were observed with PDK 40 and 53. PDK 11 and the parent seed S16681 had approximately the same plaque size.

As stated above, all animals were vaccinated with 0.5 ml of above vaccines, with the exception of PDK-11 (that was diluted 1:18) and the parent seed (that was diluted 1:8) as previously agreed, before inoculation. Diluent used was sent by WRAIR.

After vaccination, animals were bled for 14 consecutive days. As agreed, these samples were sent to WRAIR for viremia studies. On days 30 and 58 post vaccination (May 22, and June 20, 1991) animals were bled and PRNTs against Dengue-2 (S16681 strain) were performed.

Results of these tests are presented in Table III (enclosed).

Future plans: Future plans call for the evaluation of other Dengue vaccine candidates, as described in the original application.

Man hours incurred: As described on original application for this time period.

Edmundo Kraiselburd, Ph.D.

Edmundo Kraiselburd, Ph.D.
Professor and Principal Investigator

TABLE III

ANTIBODY RESPONSE OF MONKEYS INOCULATED WITH DIFFERENT
DEN-2 (S16681) VACCINE CANDIDATES

Monkey No.	Vaccine	Vaccine Innoculum Pfu/0.5ml	N. Antibody Titers*		GMT
			day 30	day 60	
	S16681	2×10^6		1838	≥ 4608
R25			1250	4800	
R13			2560	5200	
R50			2300	3500	
H5			1550	>5120	
	PDK11	2.2×10^5		1267	≥ 4104
J15			800	4100	
R28			1280	2700	
R41			1800	5000	
J16			1400	>5120	
	PDK40	1.1×10^5		338	1652
J21			560	2560	
R35			260	2000	
J20			150	470	
J22			600	3100	
	PDK53	1.60×10^4		323	1290
R6			640	4300	
R16			330	1120	
R1			270	900	
R38			190	640	

*Virus used for PRNT: Parent seed S16681 STRAIN
GMT = Geometric mean titer