

Evaluation of the Antigenic Potency of Biken JEV Vaccine in Adults

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OBJECTIVE: To determine the antigenic potency of the killed Japanese encephalitis vaccine (Biken purified mouse brain) in a group of young adult Peace Corps Volunteers (PCV's) in Thailand.

INTRODUCTION: The background and experimental design of this collaborative study between the U.S. Peace Corps in Thailand and SMRL was covered in last year's Annual Report.

PROGRESS: Three different lots of vaccine (A,B,C) have now been given to 3 groups of volunteers, one lot per group. The experimental design is outlined in Table 1. Serological tests have been performed on most of the serum up to and including the 7-12 week post-booster serum. The results of the HI and PRNT tests against JEV (Nakayama strain) are summarized in Tables 2-5. The average conversion rate in volunteers without detectable JEV antibody in preimmunization serum determined 4-6 weeks following primary immunization for the 3 vaccine lots was 20% by HI and 17% by PRNT. The total percentage of PCV that had converted after 3 immunizations, including booster, was 31% by HI and 22% by PRNT.

The results indicate that this killed JEV vaccine lacks antigenic potency in American adults.

A question can be raised concerning the sensitivity of the serological tests used to measure serum antibody. An insensitive test would naturally lead to the conclusion that the vaccine lacked antigenic potency. An attempt was therefore made to determine the ability of the HI and PRNT to measure antibody in serum obtained from 3 individuals with primary inapparent JEV infections. The serum donors were 3 healthy Chiangmai Valley villagers who had no serological evidence of prior group B arbovirus infection. Their lack of prior exposure to JEV was confirmed by the detection of IgM antibody to JEV in their convalescent phase serum. As seen in Table 6, the HI and PRNT titer levels were similar in the convalescent serum of all 3 subjects, and it is clear that the PRNT can detect antibody rises after inapparent natural infections. Moreover titers in 2 individuals are reasonably high (C. 1:40) using the 2 serological tests.

The collection of 7 month post booster serum is complete but the 12 month collection is pending.

MOUSE POTENCY TEST: The antigenic potency of the Biken vaccine (lot B & C) was measured further by a standard mouse potency test. The Japanese NIH method was employed. Groups of mice were inoculated IP every other day for a total of 4 injections with serial dilutions of vaccine. Seven days after the last inoculation of vaccine, the mice were challenged intracerebrally with JEV (strain BKM-984-70), 150-200 mouse LD₅₀/0.03 ml/mouse. Control groups of mice given I.P. saline were similarly challenged. The standard dose of vaccine affording 50% survival (the minimal immunizing dose (MID)) should be 0.02 cc of vaccine or less. This dose is nearly equal to the 1/32 dilution of vaccine used in this test. The results revealed that the MID for vaccine lots B & C were both 0.0625 cc which corresponds to a vaccine dilution of 1/8. Thus two lots of Biken vaccine used in this study are but 1/3 to 1/4 the minimal acceptable potency for a JEV vaccine. If the 7 and 12 month antibody titers show no striking improvement over the low conversion rate in earlier sera, then the vaccine tested may not be suitable for use in American adults.

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Table 1.
Experimental Design Biken Vaccine Potency Test in PCV

<u>Immunization</u>	<u>Bleeding</u>	<u>Time following onset</u>	<u>Vaccine lot</u>
1st dose	acute	0 days	A, B, C
2nd dose		7 days	A, B, C
Booster dose	Post 1° immunization	3-6 weeks	A, B, C
	Post booster	7-12 weeks	A, B, C
	Post booster	7 months	A, C
	Post booster	15 months	C

Table 2.
Biken JEV Vaccine Study: HI Test Results After Primary Immunization

<u>PCV group</u>	<u>Vaccine lot</u>	<u>No. studied</u>	<u>No. converted^x</u>	<u>% converted</u>
00-32	A	107	18	17
33	B	11	1	10
34-35	C	91	23	25
	Totals	209	42	20%

x Acute serum titer = <1:10
Convalescent serum titer = ≥ 1:20 (3-6 weeks)

Table 3.
Biken JEV Vaccine Study: PRNT Results after Primary Immunization

<u>PCV group</u>	<u>Vaccine lot</u>	<u>No. studied</u>	<u>No. converted^(x)</u>	<u>% converted</u>
00-32	A	57	9	15
33	B	11	2	20
34*	C	40	7	15
	Totals	108	18	17%

(x) Acute serum = <1:10
 Convalescent serum titer = > 1:10 (3-6 weeks)
 * Group 35 sera not yet titered

Table 4.
Biken JEV Vaccine Study: HI Test Results after Booster Immunization

<u>PCV group</u>	<u>Vaccine lot</u>	<u>No. studied</u>	<u>No. converted^(x)</u>	<u>% converted</u>
00-32	A	50	13	26
33	B	11	1	10
34-35	C	81	31	38
	Totals	142	45	31%

(x) Acute serum titer = <1:10
 Convalescent serum titer = \geq 1:20 (7-12 weeks)

Table 5.
Biken JEV Vaccine Study: PRNT Results after Booster Immunization

<u>PCV group</u>	<u>Vaccine lot</u>	<u>No. studied</u>	<u>No. converted^(x)</u>	<u>% converted</u>
00-32	A	31	5	16
33	B	11	3	30
34*	C	39	10	24
	Totals	81	18	22%

(x) Acute serum titer = 1:10

Convalescent serum titer = > 1:10 (7-12 weeks)

* Group 35 sera not yet titered

Table 6.
Serological Test in Primary* Inapparent JEV Infection

<u>Villager</u>	<u>Serum No.</u>	<u>Date</u>	<u>Reciprocal Antibody titer vs JEV</u>	
			<u>HI</u>	<u>PRNT</u>
JE-C-6c	44586	9 June 70	0 ^x	0 ^x
	46037	14 August 70	80	60
JE-L-121	44709	11 June 70	0	0
	44710	23 June 70	40	20
JE-L-11a	44762	15 June 70	0	0
	44763	29 June 70	80	105

* Primary Infection confirmed by detection of IgM HI Antibody to JEV in the convalescent phase serum.

x 0 = <1:10