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c. *Response in Children to an Experimental Inactivated Virus Vaccine Grown in Hamster Diploid Cell Culture**

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Following our experience with inactivated Japanese Encephalitis (JE) vaccine grown in hamster diploid cell culture in laboratory animals including a number of experiments demonstrating protection from intranasal infection in monkeys,[1,2] and thorough safety testing, we have undertaken exploratory immunization in children in Taiwan. We started with children with cerebral palsy or severe mental defect and proceeded carefully stepwise to test a total of 127 children.

Vaccines: The method used for cell culture vaccine preparation has been described.[2] The vaccines employed consisted of 2 lots, number 136 and 146. Both of them prepared in HS-5 strain of hamster diploid cell culture infected with JEO strain of JE virus. The virus harvest was clarified by centrifugation at 8,000 RPM for 30 minutes and inactivated for 3 weeks at 4 C with 1:2,000 formalin. A lot (No. 158) of imported commercial mouse brain vaccine was purchased from local market and was used 8 months prior to expiration date.

Vaccine Volunteers: A total of 127 children have now received experimental inactivated cell culture vaccine. Those who did not receive a complete series of vaccinations or from whom paired sera were not available were excluded from this study. They were divided into 4 groups and description of the volunteered children is presented in Table I. The first group of children to receive the vaccine came from an institution for foundlings and all suffered from cerebral palsy or severe mental retardation. These children were poorly nourished and 9 of them lived in a hospital temporarily for special nursing care. All other subjects in the study were healthy normal children. The age of the children in Study I was not known. Eighty per cent of the rest of the children

Table 1

Description of Volunteered Children

A	Group	Support			No.		Age range		Health	
	I	Institution			21		2–13 years		Cerebral palsy or severe mental retardation	
	II	Children's Home A			17		1–6 years		Normal	
	III	Children's Home B			19		2–7 years		Normal	
	IV	Pediatric OPD			70		2–10 years		Normal	
B	AGE DISTRIBUTION (Study II–IV)									
	Age (Yr.)	1	2	3	4	5	6	7	8+	Total
	No. of vaccines	2	25	28	15	18	9	6	3	106
C	SEX RATIO OF THE VOLUNTEERS (Study II–IV)									
	Male	62								
	Female	44								

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were between 2 and 5 years of age. Consent for immunization was obtained from the guardian or parents of the children and those with initial high antibody titer were dropped from the study. In addition to the children receiving the cell culture vaccine there were 9 children in Study II who were given the commercial mouse brain vaccine and 13 children in Study III who received placebo.

Immunization Schedule: All 127 children received 3 doses of 1 ml of the fluid cell culture vaccine before onset of the annual epidemic of encephalitis. Each dose was given 2 weeks apart, except in Study III, where 4 and 6 weeks separated the 3 injections. After inoculation, children were carefully observed for immediate side reactions and for 48 hours for febrile or later reaction. The four studies were carried out sequentially and we proceeded only when no adverse reactions were observed at any step.

Serological Study: Blood specimens were obtained from all children prior to immunization and 3 to 4 weeks after the last immunization. In Study III, sera were also available 4 weeks after the second vaccine injection. Neutralizing antibody was determined in these sera by a metabolic inhibition test carried out in hamster diploid cell culture. The metabolic inhibition test is carried out in tubes. About 6 days after inoculation of virus-serum mixture, when 10^{-1} dilution of the challenge virus showed complete cytopathic effect in simultaneous titration, all cell culture tubes were refed with fresh medium at pH 7.8 and returned to the 36C incubator. The results were read 24 hours later. Change in color of the medium to yellow indicated the presence of antibody. The titer obtained from this test was similar to that obtained by microscopic observation of the tubes for cytopathic effect and easier to read.[3]

Potency and Titration of the Vaccine Lots: Our methods for the intracerebral mouse protection test[4] and the monkey protection test[5] have been reported. Table II shows the

TABLE II

Titration of the Vaccine Lots Used for Human Immunization

Vaccine lot	Virus titer before inactivation		Mouse potency test	
	LD_{50}/ml.	HA/0.5 ml.	MED_{50}	IC challenge virus LD_{50}
136	$10^{8.4}$	384	7.9	38
146	$10^{9.3}$	384	6.9	38

Both lots had 1% human albumin in the final medium.

results of titration of the vaccine lots 136 and 146. Although the suckling mouse infectivity titer of lot 136 was lower than usual, the hemagglutination titer and the mouse potency test results were good. Both vaccines met the minimal requirement in the intracerebral mouse potency test established by the Japanese National Institutes of Health for mouse brain vaccine. The commercial mouse brain vaccine was also examined by the mouse potency test against intracerebral challenge with low passage HV1 strain. The effective dilution protecting 50% of the mouse from intracerebral challenge dose of 31 LD_{50} was 2.7. Cell culture vaccine lot 146 and mouse brain vaccine lot 158 were inoculated into groups of 5 monkeys. All monkeys developed neutralizing antibody of 1:64 or greater after 4 doses of the cell culture vaccine, whereas none of the monkeys receiving the same dose of the mouse brain vaccine, except one which had preantibody of 1:16, developed 4-fold antibody rise.

Safety Tests of the Hamster Diploid Cell Strain (HS-5) and Vaccines: Both the HS-5 diploid

cell strain and the lots 136 and 146 of cell culture vaccine were safely tested by methods possible in our facilities, modified from those required for mouse brain vaccine in Japan[6] and those suggested by Hayflick *et al*[7] for diploid cell culture vaccine. Bacteriological sterility of the vaccine was confirmed by testing virus harvest before and after formalin treatment in blood agar plates, thioglycolate tubes, PPLO agar plates and Ogawa's medium (for Mycobacterium). The absence of exogenous cytopathic agents were demonstrated by testing vaccine and cell suspension in routine tissue culture systems and laboratory animals. Tissue cultures employed were human diploid, primary monkey kidney and Hep-2 cell cultures, each observed for 14 days with one blind passage. Two litters of suckling mice and 10 of weanling mice inoculated both intracerebrally and intraperitoneally were normal for 3 weeks. Groups of young hamsters were inoculated into the cheek pouch and intradermally/subcutaneously with a suspension of 10^6 viable cell of different passages of the HS-5 strain and 0.5 ml of vaccine. No induration or tumor formation was demonstrable during 6 months observation. Five guinea pigs were inoculated intraperitoneally with 5 ml of the vaccine. All animals gained weight during a week's observation. Chromosomes of the HS-5 cell strain were studied every 5–10 passages according to the method described by Tijo *et al*.[8] with slight modification. After 24 hours of cell growth on glass cover slips in Leighton tubes, colchicine was added to make a final concentration of 5 microgm/ml and incubation continue for 5 hours. The cells were, after treatment with hypotonic solution (0.563%K Cl) at room temperature for 15 minutes, fixed with 3:1 of alcohol and acetic acid mixture, air dried and stained with Giemsa. The cell strain remained diploid through at least 35 passages, the last one tested.

The nitrogen content of the vaccines was tested by Dr. P.C. Huang, Department of Biochemistry, National Taiwan University College of Medicine. Kjedahl method was used. For example, the nitrogen of the medium used for vaccine lot 146 (mostly human albumin) was 1.94 mg/ml and of the virus harvest fluid prior to formalin treatment was 1.92 mg/ml. The net nitrogen content attributable to the virus and cell was 0.02 mg/ml.

Serological Response to Vaccination: Tables III, IV, V and VI show the antibody response to vaccine in the 4 studies. Table III shows that there was antibody response in the undernourished cerebral palsied children, but this antibody response to vaccine 136 was poorer than observed in normal children. In Study II shown in Table IV, all children given vaccine 136 had 4-fold or greater neutralizing antibody response, while 6 out of 9 given a particular lot of mouse brain vaccine failed to show 4-fold rise in antibody. Tables V and VI show the results of Studies III and IV with vaccine 146. A longer interval was used between injections in Study III than in the other studies. Table V shows a relatively low antibody response 4 weeks after 2 injections of vaccine. Antibody

TABLE III

STUDY I: Antibody Response in 21 Institutionalized Children with Cerebral Palsy Following Three 1 ml. Inoculations at 2 Week Intervals of Lot 136 Inactivated JE Vaccine

MI antibody titer	MI antibody titer 4 weeks after				
	<4	4	8	16	<32
<4	2	4	3	6	4
4					
8					2

MI = metabolic inhibition, cell culture tube neutralization test.

TABLE IV

STUDY II: Antibody Response in 26 Healthy Children Following Three 1 ml. Inoculations at 2 Week Intervals of Either Hamster Diploid Cell Culture Vaccine Lot 136 or Japanese Commercial Mouse Brain Vaccine

MI antibody titer before vaccine	MI antibody titer 4 weeks after last vaccine injection				
	<4	4	8	16	<32
Lot 136					
<4			4	5	5
4				1	2
Mouse brain vaccine					
<4			3		
4			3	1	2

TABLE V

STUDY III: Antibody Response in 19 Children Following Three 1 ml Injections of Lot 146 Cell Culture JE Vaccine Separated by 4 and 6 Weeks

	MI antibody titer						
	<4	4	8	16	32	64	≥128
Prior to vaccine	17	1	1				
4 wks. after 2 vaccine injections	7	4	2	2	4[1]		
3 wks. after 3 vaccine injections		1		7	5	4	2[1]

[1] includes both children with low titer antibody prior to vaccine.

TABLE VI

STUDY IV: Antibody Response in 70 Children Brought to the Pediatric OPD For Three 1 ml Injections, 2 Weeks Apart, of Experimental Cell Culture JE Vaccine Lot 146

MI antibody titer before vaccine	MI antibody titer 4 weeks after last vaccine injection				
	<4	4	8	16	≤32
<4			9	34	25
4					1
8					1

response after the third vaccine injection was excellent. The 13 children that received placebo showed no antibody response. In Table VI, the antibody response in the 70 children inoculated in the pediatric OPD was also good, none failing to show at least 4-fold neutralizing antibody response. Of the 106 normal children given 3 injections of vaccine lots 136 or 146 only one failed to demonstrate 4-fold or greater rise in antibody.

Side Reactions to Vaccine: No adverse reaction was observed in most of the vaccinees except that a few children in Studies I and IV had measurable rise of body temperature following vaccination. No febrile reactions were detected in Studies II and III. In Study I, febrile reactions were recorded in 6 out of 63 doses of the vaccine. All these reactions occurred to 4 of the hospitalized children. In Study IV, rise of body temperature was recorded in 12 out of 210 doses of the vaccines. During this study period, febrile upper respiratory infection was prevalent in Taipei areas where the children lived. In fact, 39 out of 143 apparently healthy volunteers were rejected from this study on account of high

body temperature detected prior to the first immunization. Many of these febrile children were siblings of the volunteers accepted for vaccination. There were also more than 10% of the volunteers with elevated temperature prior to the second and third injection. Therefore, most, if not all, of the 12 febrile episodes may have been unrelated to the vaccine. The only local reaction was some pain and burning at the time of injection.

SUMMARY

Formalin inactivated Japanese encephalitis virus vaccine grown in hamster diploid cell culture was tested in 127 children in Taiwan. They included 21 handicapped and malnourished children and 106 normal children.

Only 71% of the handicapped children responded with 4-fold rise of neutralizing antibody to 3 doses of 1 ml of the vaccine, while all except one of 104 normal children had at least a 4-fold antibody rise.

No significant adverse reaction was noticed after vaccination.

REFERENCES

1. Lee, G.C.Y., Grayston, J.T. and Wang, S.P.: Protective Studies in Mice and Monkeys with an Inactivated Japanese Encephalitis Virus Vaccine Grown in Hamster Diploid Cell Culture. *Proc. Soc. Exp. Biol. Med.*, **125:** 803–808, 1967.
2. Grayston, J.T. and Lee, G.C.Y.: Development of an Inactivated Japanese Encephalitis Virus Vaccine Grown in Embryonic Hamster Diploid Cell Culture. (IV D3c, This book.).
3. Lee, G.C.Y. *et al.*: To be published.
4. Lee, G.C.Y. and Grayston, J.T.: Cross Protection Test with Japanese Encephalitis Vaccine in Mice and Monkeys. (III A1, This book.)
5. Lee, G.C.Y. and Grayston, J.T.: Monkey Intranasal Challenge against Japanese Encephalitis Vaccine. (IV B2g, This book).
6. *Minimum Requirements of Biologic Products.* Ministry of Health and Welfare, Japanese Government Publication, 1958. Amended by Notification No. 329, Oct., 1961.
7. Hayflick, L., Plotkin, S.A., Norton, T.W. and Koprowsky. H.: Preparation of Poliovirus Vaccines in a Human Fetal Diploid Cell Strain. *Am. J. Hyg.*, **75:** 240–258, 1962.
8. Tijo, J.H. and Puck, T.T.: Genetics of Somatic Mammalian Cells, II. Chromosomal Constitution of Cells in Tissue Culture. *J. Exp. Med.*, **108:** 259–268, 1958.