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STUDIES ON LIVE RUBELLA VACCINE. III. FIELD TRIALS WITH VACCINES ATTENUATED BY SERIAL PASSAGES IN PRIMARY PIG KIDNEY CELLS

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SUMMARY Live attenuated rubella virus vaccines at different passage levels in primary pig kidney (PK) cells were administered to a total of 516 susceptible children and results were evaluated clinically and serologically.

The progress of attenuation was seen clinically after serial passage in PK cells. All vaccines tested were immunologically potent, and did not cause any contact infection to 14 control siblings.

The low temperature-variant character of these vaccines and the usefulness of PK cells for rubella virus vaccine production were discussed.

INTRODUCTION

Previous reports from our laboratory described the results of field trials with two experimental vaccines (ME6 and ME11) (Okuno et al., 1968; Minekawa et al., 1968) produced in chick amniotic cavity. Using this method it was difficult to obtain a high enough virus yield and titers often fluctuated from one passage to another, although some extent of attenuation was achieved by serial passage in chick amnion.

To obtain a higher yield of virus we tested other hosts in place of chick amnion. We found that PK cells yielded enough virus for vaccine production, while Horta-Barbosa and Warren (1969) reported their low sensitivity to rubella virus.

The field trials reported here were undertaken to compare the grades of attenuation of several vaccines at different passage levels in PK cells. Attenuation was studied clinically and immunologically, and the absence of viral communicability was confirmed using siblings as contacts.

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MATERIALS AND METHODS

1. *Vaccine preparation*

Chick amnion-adapted Matsuura strain (Okuno et al., 1968) was serially passaged in primary PK cells at 33 C or sometimes at 27 C for lower temp passage (LP). Tissue culture fluids at appropriate passage levels were used as experimental vaccines after freeze-drying. The vaccines were named according to their passage histories. For example ME17PK8 is vaccine of the Matsuura strain passaged 17 times in chick amnion and 8 times in PK cells. The passage histories and titers of the vaccines are shown in Fig. 1.

Vaccine virus was titrated by measuring plaque-formation in RK13 cells or by the interference method (Okuno et al., 1956) in primary African green monkey kidney (GMK) cells with ECHO-11 virus.

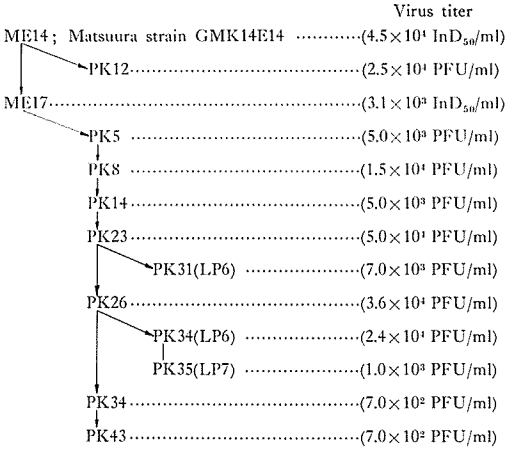

FIGURE 1. *Passage history of vaccines in PK cells. They were abbreviated, for example, as PK31 (LP6): Matsuura strain, passaged in PK cells 25 times at 33 C and 6 additional times at 27 C following 14 passages in GMK cells at 36 C and 17 passages in chick amniotic cavity at 32 C.*

2. *Vaccines*

Healthy infants aged between 1 and 5 years in an open community were given a single dose of 0.5 ml of vaccine preparation by subcutaneous injection into the upper arm. They were bled for antibody titration before and 4 weeks after administration with vaccine. Vaccinees whose body temp rose to 37.5 C or higher between week 1 and 3 after vaccination were evaluated as febrile. Only data on susceptible children are described in this paper.

3. *Serological test*

The serological response was measured by a modification of the HI method described by Stewart et al. (1967) in a microtiter tray (Cooke Engineering Co., Va., U.S.A.) using 0.33% goose erythrocytes in PBS (−) (phosphate buffered saline without divalent cations), pH 7.2. To eliminate nonspecific hemagglutination-inhibitor(s), 0.1 ml of sera were diluted 1:4 with 0.3 ml of PBS, pH 6.4, containing 0.4% bovine albumin (Fraction V, Armour Pharmaceutical Co., Ill., U.S.A.) and treated with an equal volume of 25% kaolin (acid-washed, Fischer Scientific Co., N. J., U.S.A.) in PBS for 20 min at room temp and then by adsorption with 0.05 ml of 50% goose erythrocytes in PBS(−), pH 7.2. Serum samples which had no HI activity at 1:10 dilution were regarded as seronegative or susceptible.

RESULTS

Live rubella vaccines, originating from the Matsuura strain adapted to chick amnion (Okuno et al., 1968), at different passage levels in PK cells were administered to 516 susceptible children and 342 of them were bled to test the immunogenic potency of the vaccines.

The seroconversion rate together with geometric mean antibody titer and the clinical reactions to PK12 through PK26 are shown in Tables 1 and 2, respectively. Those of 5 other vaccines (after more than 30 passages in PK cells) are summarized in Table 3. Almost all vaccines were found to be immunologically potent and their seroconversion rates were all 100% except that of ME17PK34 (LP6) lot #2 vaccine was 93%. Clinically, a rash appeared in 31.8 to 16.0% of the children vaccinated with PK12 through PK26 vaccines (Table 2), but its frequency decreased gradually with vaccines PK31 (LP6) to PK43 (Table 3). Passage at lower temp (LP) (27 C) resulted in decrease in the frequency of rash. The frequency of a febrile reaction (above 37.5 C) also decreased and was very different with PK8

and with PK14 (Table 2). Highly passaged vaccines (higher than the 30th) including LP seemed to have the character of adequately attenuated live vaccine (Table 3).

The ratios of antibody responses were 100% with all the vaccines except ME17PK34 (LP6) lot #2 and the geometric mean value of the HI antibody titer did not change much on increasing the passage number (Table 1, 3).

Tests on the contagiousness of the vaccines by measuring seroconversion of the contacts showed that even the low passaged vaccines,

TABLE 1. *Summary of HI antibody responses to five experimental live rubella vaccines*

Vaccine (PFU/dose)	Seroconv. rate (%)	GMT of post HI Ab (2^n)
ME14PK12 (6.3×10^3)	15/15 (100)	7.4
ME17PK8 (3.8×10^3)	15/15 (100)	7.1
ME17PK14 (1.3×10^3)	22/22 (100)	7.0
ME17PK23 (1.3×10^4)	36/36 (100)	7.3
ME17PK26 (1.8×10^4)	49/49 (100)	7.6
Total (average)	137/137(100)	7.3

TABLE 2. *Summary of clinical reactions in children vaccinated with five experimental live rubella vaccines*

Vaccine (PFU/dose)	Rate of clinical reaction		Arithmetic mean value of			
	Rash (%)	Fever (%)	Day of onset of Rash	Day of onset of Fever	Max. fever (C)	Durat. of fever (Day)
ME14PK12 (6.3×10^3)	7/22 (31.8)	6/22 (27.3)	11.8	10.5	38.0	1.5
ME17PK8 (3.8×10^3)	6/36 (16.7)	6/36 (16.7)	10.2	9.0	38.3	1.2
ME17PK14 (1.3×10^3)	5/29 (17.2)	3/29 (10.3)	12.6	9.7	37.9	1.0
ME17PK23 (1.3×10^4)	10/56 (17.9)	4/56 (7.1)	11.4	10.3	37.9	1.3
ME17KP26 (1.8×10^4)	12/75 (16.0)	10/75 (13.3)	9.6	11.6	38.4	1.7
Total (average)	40/218 (18.4)	29/218 (13.3)	11.1	10.2	38.1	1.3

TABLE 3. *Summary of clinical reactions and antibody responses to PK-passaged live rubella vaccines*

Vaccine (PFU/dose)	Rate of clinical reaction		Seroconv. rate (%)	GMT of post HI Ab (2^n)
	Rash (%)	Fever (%)		
ME17PK31 (LP6)				
lot#1 (5.0×10^3)	1/16 (6.3)	2/16 (12.5)	16/16 (100)	7.0
lot#2 (3.5×10^3)	1/25 (4.0)	2/25 (8.0)	25/25 (100)	6.4
lot#3 (4.4×10^3)	1/37 (2.7)	5/37 (13.5)	25/25 (100)	6.8
ME17PK34 (3.5×10^2)	3/28 (10.7)	3/28 (10.7)	8/8 (100)	7.8
ME17PK34 (LP6)				
lot#1 (4.4×10^2)	3/57 (5.3)	4/57 (7.0)	38/38 (100)	6.7
lot#2 (1.2×10^4)	4/61 (6.6)	6/61 (9.8)	39/42 (93)	6.3
ME17PK35 (LP) (5.0×10^2)	0/36 (0.0)	2/36 (5.6)	26/26 (100)	6.7
ME17PK43 (3.5×10^2)	2/38 (5.3)	1/38 (2.6)	25/25 (100)	6.6
Total (average)	15/298 (5.0)	25/298 (8.4)	202/205 (99)	6.8

TABLE 4. *Result of communicability test on experimental live rubella vaccine ME14PK12 in siblings*

Family no.	Sibling	Clinical reaction	Virus excretion	HI Ab titer		
				Before	After 6 wks	10 wks
1	1) M. K.[a]	—	−	<×10	×160	NT[c]
	2) M. K.[b]	—	NT	<×10	<× 10	NT
2	1) A. O.	—	−	<×10	NT	×160
	2) T. O.	—	NT	<×10	NT	<× 10
3	1) J. T.	Rash	−	<×10	×160	×320
	2) A. T.	—	NT	<×10	<× 10	<× 10
4	1) E. Y.	Fever	+	<×10	× 80	×160
	2) A. Y.	—	NT	<×10	<× 10	<× 10
5	1) K. T.	Lymphadenopathy	+	<×10	×160	NT
	2) N. T.	—	NT	<×10	NT	<× 10
6	1) Y. K.	—	−	<×10	×160	×160
	2) T. K.	—	NT	<×10	<× 10	<× 10
7	1) T. K.	—	+	<×10	×160	NT
	2) N. K.	—	NT	<×10	<× 10	<× 10
8	1) Y. T.	—	NT	<×10	NT	×160
	2) S. T.	—	NT	<×10	NT	<× 10

[a] Vaccinee. [b] Contact. [c] Not tested.

TABLE 5. *Result of communicability tests on experimental live rubella vaccines, ME17PK8 and ME17PK14, in siblings*

Vaccine	Family no.	Sibling	Clinical reaction	HI Ab titer			
				Before	After 6 wks	7 wks	8 wks
ME17PK8	1	1) N. F.[a]	—	<×10	×80	NT[c]	NT
		2) K. F.[b]	—	<×10	<×10	NT	NT
	2	1) F. K.	Fever	<×10	×80	NT	NT
		2) T. K.	—	<×10	<×10	NT	NT
	3	1) M. T.	Fever	<×10	NT	×160	×160
		2) H. T.	—	<×10	NT	<× 10	<× 10
	4	1) S. K.	—	<×10	NT	×160	NT
		2) M. K.	—	<×10	NT	<× 10	NT
ME17PK14	1	1) S. A.	—	<×10	NT	NT	×320
		2) T. A.	—	<×10	NT	NT	<× 10
	2	1) Y. K.	—	<×10	×80	NT	NT
		2) E. K.	—	<×10	<×10	NT	NT

[a] Vaccinee. [b] Contact. [c] Not tested.

PK8, PK12 and PK14, did not cause any contact infection of siblings who were in close contact with the vaccinees (Table 4, 5). Even contact with vaccinees who showed clinical symptoms such as a rash and/or fever, did not result in any seroconversion to rubella virus.

Fig. 2 shows the low temperature-variant character of the vaccines. At ordinary temp (37 C) the viruses do not grow to high titer. This may be one reason why the vaccine viruses do not grow well at the body temp of the vaccinees and so do not cause contact infection.

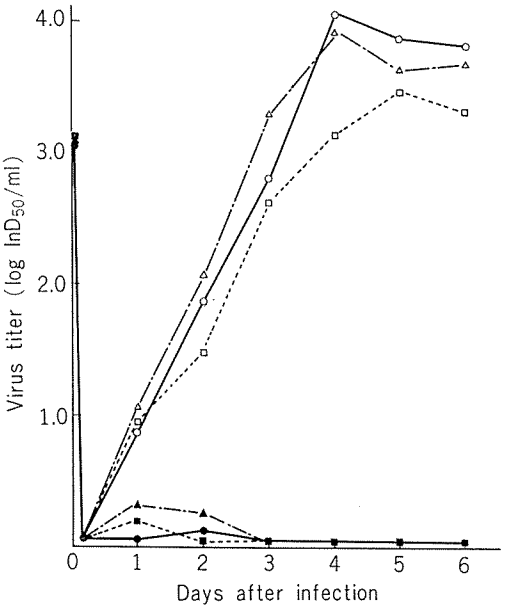

FIGURE 2. *Growths of live rubella baccine viruses in PK cells were compared at 37 C and 32 C. Virus titration was carried out in GMK cells by the interference method as described in MATERIALS AND METHODS.* ○———○, *ME17PK23;* △—·—△, *ME14PK12;* □·····□, *ME17PK8 at 32 C.* ●———●, *ME17PK23;* ▲—·—▲, *ME14PK12;* ■-----■, *ME17PK8 at 37 C.*

DISCUSSION

As previously reported, two experimental live rubella vaccines, ME6 and ME11, showed 100% seroconversion rate in vaccinees. However, although some attenuation was achieved by serial passage in chick amnion, their attenuation was not complete and the virus titers often fluctuated.

Unlike these vaccines, PK-passaged vaccine had a stable virus titer with little fluctuation during passages, as shown in Fig. 1. Furthermore, the virus yield was high and experimental live vaccine could be produced easily. These vaccines were immunologically potent, and the geometric mean antibody titer of the vaccinees did not decrease with progress of attenuation. This indicates the stability of the viral immunogenicity.

Clinically a rash was observed in 16.0 to 31.8% of the vaccinees who received "lower passage" vaccines (less than 26 passages), and in less than 10% of those who received "higher passage" vaccines (more than 30 passages). This suggests that some event necessary for attenuation occurred around the 30th passage. For attenuation serial cultivation at lower temp was more effective than at higher temp. For example, PK 34 vaccines passaged at 33 C and 27 C caused rashes in 10.7 and 5.3% of the vaccinees, respectively.

The frequency of a febrile reaction also became less on serial passage in PK cells, decreasing from 16.7% at the 8th passage to 10.3% at the 14th passage. No lymphoadnopathy or other clinical reactions were seen.

Tests on the communicability of vaccines have suggested that the amount of virus excreted in the throat or in the upper respiratory tract should be important in establishment of contact infection, as mentioned by Meyer et al. (1967). In the present case even when virus was detected in throat swabs, its amount may have been too small to cause contact infection even to siblings living in the same house.

It is still uncertain how long the antibody level is maintained after vaccination with PK-vaccine. However, follow-up studies on persons vaccinated with ME6 or ME11 vaccine 5 years ago showed the presence of antibody levels high enough to give protection against a rubella epidemic (Minekawa et al., unpublished data). From these data it seems probable that the antibody levels reported in this

paper will be maintained for a long time as protection against rubella epidemics.

The temperature-sensitive character of experimental vaccine viruses may have become dominant in the population during many serial passages at 33 C or lower temp. This character is expremely suitable, as the normal body temp of the vaccinees would not allow sufficient growth of the vaccine viruses for their excretion into the throat and this explains why no contact infection was observed.

Other investigators have tested primary rabbit kidney (RK) cells (Peetermans and Huygelen, 1967), primary duck embryo (DE) cells (Hilleman, 1966; Hilleman et al., 1969), primary canine kidney (DK) cells (Meyer et al., 1969), or human diploid cells (WI-38) (Plotkin, Cornfeld and Ingalls, 1965), and confirmed the usefulness of these cells. PK cells are highly sensitive to rubella virus, which makes serial passage possible even at low temp, and they are suited for vaccine production. They seem to have little viral contaminant(s) like other primary cells, such as RK, DK, DE or WI-38.

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