Arch. Inst. Razi, 1977, 29,11-32

A COMPARATIVE FIELD TRIAL OF FIVE MEASLES VACCINES PRODUCED IN HUMAN DIPLOID CELL, MRC-5

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Five batches of experimental measles vaccines were prepared with Biken-CAM, Schwarz, Sugiyama, Leningrade-16 and AIK-C strains in MRC-5 HDC. The rct 40, T50 or neurovirulence for baby hamsters of these viruses adapted to HDC were not significantly changed but the neurovirulence of the Sugiyama virus for day-old hamsters was increased after passages in HDC. Whith the exception of the AIK-C strain the size of the plaques was increased by 20 passages of virus in MRC-5 cells.

The experimental vaccines were evaluated clinically and serologically in a field trial in seronegative children in the Caspian Sea area north of Iran. It was found that: (a) the lowest clinical reactions were recorded for the AIK-C vaccine; (b) the seroconversion was high for all vaccines ranging between 95.5 and 100%; and (c) that the hemagglutination-inhibiting (HI) antibody titers of sera of the vaccinees ranged from $2^{5.2}$ to $2^{6.5}$.

INTRODUCTION

In the large-scale production of viral vaccines, the problems of heterogeneity and viral contamination of non-human primate cell cultures have been overcome by the introduction of WI-38 cells of human fetal origin which have been shown to be diploid in character (human diploid cells, HDC) by Hayflick & Moorhead (1961). The stability and integrity of this human cell strain have been well established also by other reports in the past decade (Rubin, Minecci & Tint, 1967; Andzaparidze, Rapoporte, Dzagurov, Kolchurina & Alshtein, 1968; Perkins, 1968) etc.

(*) Reprinted from journal of Biological standardization 1977 5,1-18.

It is understandable that such a new approach to cell substrates for the production of virus vaccines would take time to be accepted and at a conference held in Bethesda in November 1967, Dr Perkins observed that 'so far, no vaccine prepared in WI–38 cells has been licensed for sale, either in the U.K. or in the U.S.A. However, it is an encouraging sign to see so much work in progress in the use of these cells and we must move towards the ideal situation of establishing a "cell seed system" for our future vaccines'.

Today this excellent 'cell seed system' is applied everywhere for the manufacture of virus vaccines and it is now urgent for us to think about a candidate HDC to replace WI–38 since the stock will soon be exhausted. Accordings to a report presented in 1969 to the Committee on Cell Culture of the International Association of Biological Standardization, a new HDC developed by Jacobs, Jones & Baille (1970) and called MRC–5, compared favourably with the widely used WI–38 cell and the results of comparative studies of WI–38 and MRC–5 HDC have shown that the MRC–5 cells could be used as an alternative to the WI–38.

In the present study five attenuated strains of measles virus were adapted to MRC–5 cell cultures and the biological characterisitcs of these viruses, as well as the results of field trials of experimental vaccines produced in MRC–5 cell cultures, are discussed.

MATERIALS AND METHODS

Cell culture

MRC–5 cells were received in a 4 oz bottle at the 9th passage* and were serially passaged in stoppered 4 oz medical flat or Roux bottles using a 1:2 subculture ratio; the procedure followed was that recommended by Jacobs (1968). When the cells reached the 16th passage, they were harvested and suspended in a-medium containing 50 parts Basal Medium Eagle (BME), 20 parts calf serum, 10 parts bovine albumin, 10 parts sucrose and 10 parts dimethyl sulphoxide (DMSO). The number of cells in each ml of medium was about 2.5×10^6 and the cells were slowly cooled at a rate of about 1–2°C per min until they reached a temperature of–80°C at which time they were then immersed in liquid nitrogen for storage. A total of 76 ampoules of 'cell seed' was stored. Before the addition of DMSO, samples of the cells were removed and were tested for the presence of bacterial contaminants, including mycoplasma. All tests showed that the cells were free from contaminants.

The production of each batch of vaccine was started from a fresh ampoule of cell seed, the cells from which were grown in monolayers in BME (Grand Island Biological Co., N.Y.) supplemented with amino acids, vitamins and glu-

* The cells were kindly supplied by Mr. p. Jacobs of the National Institute for Biological Standardization and Control, Hampstead, LONDON, U. K.

tamin. The cells were passaged to the 19th passage using BME containing 10% fetal calf serum (Microbiological Associates, Bethesda, U.S.A.) whereas the subsequent passages up to the 30th employed the same medium but 10% of the local calf serum was used (produced by collecting young-calf blood from the Tehran Slaughterhouse). The unheated calf serum was sterilized through 0.22 μm Millipore fiters and was tested for the presence of bacteria, mycoplasma and bovine viruses. These sera were tested also for their ability to support the growth of HDC and it was found that 4 of 20 batches, each of 50 l of calf serum, failed this test when five successive passages of both WI–38 and MRC–5 cells were used; these lots were rejected. Cultures were subdivided on a 1:4 basis as soon as the monolayers were confluent and they were analyzed by karyology at the 29th, 30th and 31st cell doubling.

Karyologic studies

Chromosome preparations were made on the 2nd day after subcultivation (using the conventional air-dried technique, Harden & Brunton, 1965). Chromosome counts were made on 100 metaphases examined by a magnification × 100 and selected from several preparations for their good spread of chromosomes. In all counts, major chromosome abnormalities or chromatid breaks were recorded and in addition 400 metaphases were examined at a higher magnification for polyploidy. Finally, the chromosomes of two cells were photographed for the construction of the karyotype (Fig. 1).

Vero cells

These cells (Microbiolgical Associates, Bethesda, U.S.A.) were used at passages 200–300 for virus titration and plaque assays using as growth medium Earle's Balanced Salt Solution containing 0.5% lactalbumin hydrolyzate, 0.1% yeast extract, 5% calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Maintenance medium was similar to the growth medium but the calf serum was replaced by 0.2% bovine albumin.

Viruses

Five attenuated measles viruses were isolated in MRC–5 cells directly from the original vaccines and propagated to make experimental vaccines.

AIK-C vaccine lot TV 12 (kindly supplied by Dr S. Makino, of Kitasato Institute, Tokyo) was an Enders-Edmonston virus and further attenuated by Dr Makino and his colleagues (1973*a*,*b*). The virus was passed 12 times in primary sheep kidney cells at 33°C. Plaques isolated in SPF-chick embroyo cultures were cloned in CE cells and one clone was selected as vaccine seed virus and called AIK–C strain. The 7th passage virus of AIK–C strain was used for vaccine lot

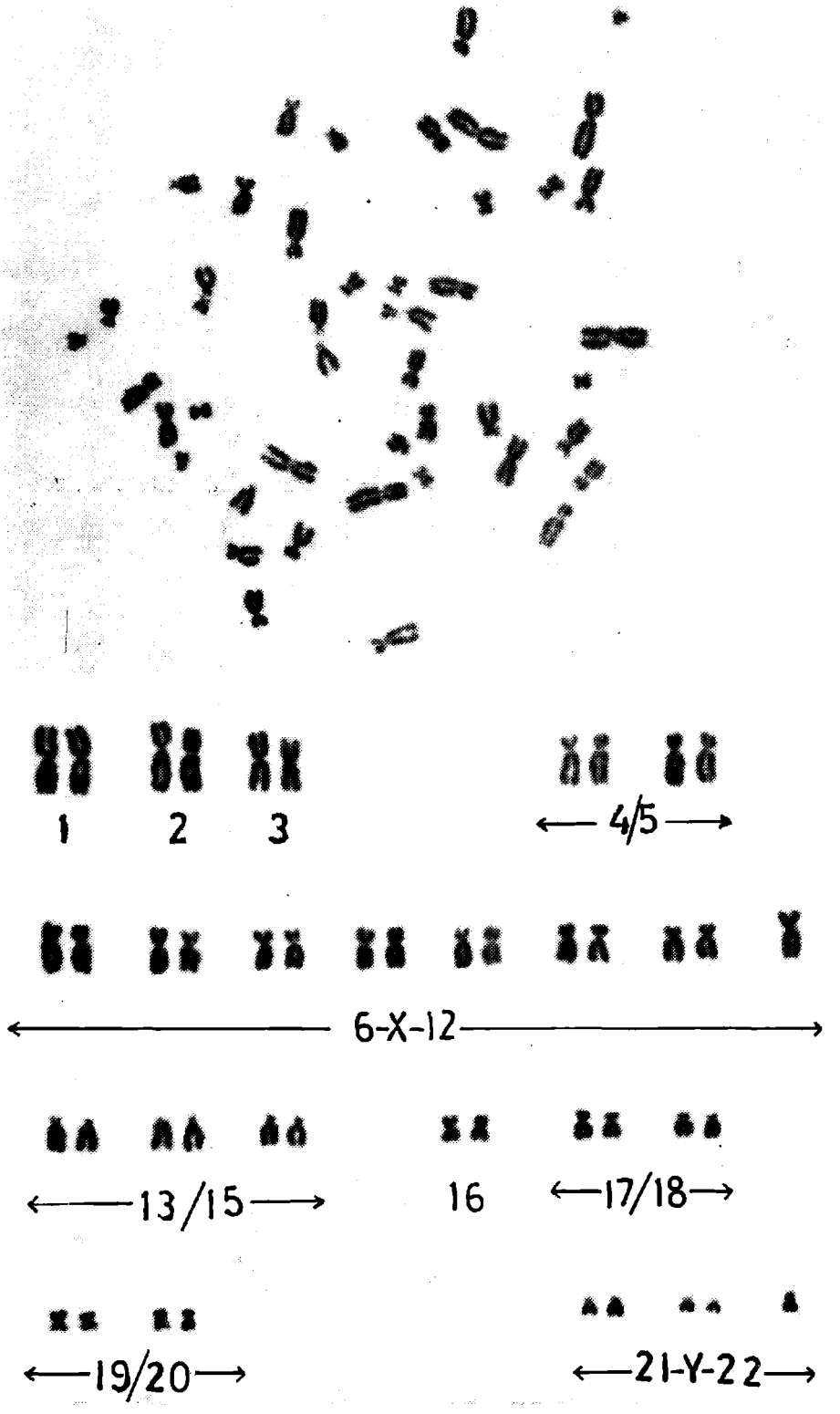

Fig. 1. Karyo type of human diploid cells MRC-5, LOT 54-2, Passage 30.

TV 12 (Makino, Sasaki & Nakamura, 1974).

Biken-CAM vaccine lot 6803 (kindly supplied by Professor Okuno and manufactured by the Kannonji Institute of Research Foundation for Microbial Diseases of Osaka University) was an attenuated strain developed by Professor Okuno and his associates from the Tanabe strain by making 3 passages in monkey kidney cell, 8 passages in human kidney cell, 90 passages in chicken embryo amnion and 35 passages in the chorioallantoic membranes of chick embryos.

Leningrade-16 *vaccine lot* 69–6 was supplied by the Moscow Research Institute of Virus Preparations.

Schwarz vaccine (*Rouvax*) *lot No. Y*0616 manufactured by the Mérieux Institute was obtained through the Ministry of Health, Tehran.

The Sugiyama strain was adapted by Matumoto, Mutai, Saburi, Fujii, Minamitani & Nakamuro (1962) to calf kidney (CK) cell. A cold variant of this strain was isolated by Myamura, Yoshizawa, Tania, Sakai,-Hashizume, Okuni, Kawara & Kimura (1971) after elution of virus from aluminium phosphate. This strain, called 5F100, was kindly supplied by Dr S. Hashizume of the Virus Department, Chiba Serum Institute, Japan. The virus was isolated from the local vaccine lot 53–10 prepared after two further passages of 5F100 virus in CK cells.

The control viruses were: (a) Edmonston virulent strain adapted 24 times in human kidney, 41 times in human amnion and 6 times in Vero cells and passed 5 times into MRC–5; and (b) the Edmonston–Zagreb strain, an attenuated virus adapted to WI–38 HDC isolated from vaccine lot 259 of the Institute of Immunology, Zagreb, in Vero cells and used for some marker studies. This strain has been passed 22 times in WI–38 (Dr Ikic, personal communication).

Vaccine production

The above strains were subcultured (0.1–0.01 p.f.u./cell) 5 times in MRC–5 at 33°C (except the Sugiyama strain which was incubated at 30°C). The final medium for virus culture was Parker 199 containing 0.2% gelatin, 50μg/ml kanamycin and 50 μg/ml Neomycin. Five experimental batches of vaccines were lyophilized and their infective titer ranged from $10^{3\cdot7}$ to $10^{4\cdot2}$ $TCID_{50}$ / dose. The safety of each batch was studied by the monkey test using *Cercopithecus aetiops* monkeys (imported from Tchad) which had been shown to be negative for measles antibody. Two monkeys were inoculated by both the intrathalamic and intraspinal routes with each vaccine; the animals were killed after 3 weeks and the brain and spinal cords were shown to be free from lesions. The absence of virulence of each strain was confirmed also by contact infection. Two monkeys were inoculated with 5000–10 000 $TCID_{50}$ of experimental vaccine and were kept in separate cages with two uninoculated monkeys free from measles antibody. After 1 month the sera of all contact monkeys were free from measles antibody.

Plaque assay

In order to study the variations in size of plaque during the serial passages of measles viruses in MRC–5 cells a plaque technique was used in Vero cells. Monolayers were washed once with Hanks' solution and inoculated with 0.2 ml. of the appropriate dilution of each virus. After incubation for 2 h at 33°C an agar overlay was added (BME, without phenol red, supplemented with 3% calf serum and 1% agarose) and after incubation of cultures at 33 or 36°C for 5 days, a second agarose overlay (containing 0.008% neutral red) was added; the plaque sizes were measured 2 days later. The variations in plaque size (S marker) were studied fo each virus after 5 and 20 passages in MRC–5 cells and the plaques were grouped into three types:

(1) S–: The average size of the majority of the plaques was $<$0.4 mm.

(2) S$\pm$: the average size of the plaques was between 0.4 and 0.6 mm.

(3) S+: the average size of the plaques exceeded 0.6 mm.

Marker tests

The rct 40 and T50 marker of viruses were studied following the procedure of Khozinski, Seibil, Pantelyeva, Mazurova & Novikova (1966). Briefly, in the rct 40 the test virus was titrated in parallel at two temperatures (35 and 40°C) and each dilution of virus was inoculated into four tubes of monolayer cell cultures. For the determination of the T50 marker the viruses free from cell debris were divided into two parts only, one of which was heated at 50°C for 15 min. Both the heated and unheated samples were titrated for virus content, the final reading of CPE of which was made after 7 days' incubation.

Hamster inoculation

One-day-old golden Syrian hamsters of a local strain were used for the study of the neurovirulance of the measles attenuated strains isolated directly from the vaccines and before adaptation to MRC–5 cells, in this way the neurovirulence of these original strains was compared with the respective viruses after each series of five passages in MRC–5 cells. The animals were inoculated into the left posterior of the brain with 0.02 ml of undiluted viruses and for each virus two litters, each with an average of six babies, were used.

Virus recovery

The brains of baby hamsters, regardless of the presence or absence of signs of illness, were removed 7 days after inoculation. All brains of each group receiving the same virus were mixed and emulsified to make a 10% (w/v) suspen-

sion in maintenance medium. The suspension was centrifuged at low speed and the supernate was inoculated into Vero cells for virus recovery; each dilution was inoculated into four tubes of Vero cell and the last reading of CPE was made 7 days after incubation.

Study population

Several villages in the Caspian Sea area north of Iran were selected for this study. Children of these villages had never been vaccinated against mealses and no natural outbreak of measles had been recorded during the past 12 months. The five experimental vaccines as well as the Rouvax (Schwarz strain) which was used as the control were coded by a number which was not disclosed before the end of the field trial. Healthy children, aged 10 months to 6 years, evenly distributed between the two sexes and without a known history of measles infection were vaccinated in each village by the vaccines. Vaccination was performed by a physician aided by several public health technicians. All information was recorded by this trained medical personnel on a card having a code number of the vaccine. The daily temperature from the 7th-15th day after immunization and significant reactions were recorded.

Serological testing

In order to evaluate the antibody response to vaccination, blood samples were collected immediately before immunization and 30 days later. The blood was collected from finger pricks as previously described (Mirchamsy, Nazari, Stellman & Esterabady, 1968) and the filter paper discs were minced and soaked overnight in the cold in 0.8 ml of saline. The tubes were then centrifuged and the clear serum extracts were heated at 56°C for 30 min, mixed with an equal volume of a 25% kaolin suspension (w/v) for 20 min at room temperature with occasional shakings to remove the non-specific inhibitors and then mixed with a 50% suspension of vervet monkey red blood cells for 1 h at room temperature to remove the non-specific hemagglutinins. A two-fold dilution of 0.025 ml (1:8) of this already-diluted treated serum was mixed with 0.025 ml of the measles virus hemagglutinin (8 HA units/0.05 ml prepared by the Norrby (1962) technique) in a microtiter plate. The plates were kept at 37°C for 1 h and then to each mixture was added 0.05 ml of a 0.5% suspension of vervet monkey red blood cells; positive and negative control sera were included daily in each test. The results were read after incubation at 37°C for 2 h and an additional reading was made after standing overnight in the cold room.

RESULTS

Characteristics of MRC-5 cells

It was found that MRC–5 cells replicate more rapidly than WI–38 cells when incubated under similar conditions. They tolerate the environmental factors much better and when a split ratio of 1:2 was applied the monolayers were confluent within 2–3 days compared with the 4 days normally required by WI–38.

The karyological findings of the MRC–5 HDC (see Table 1) were well within the recommended minimal requirements for karyological abnormalities, as outlined by the Cell Culture Committee (1974).

Adaptation of attenuated measles virus to MRC-5 cells

All vaccine strains used in the study have been well adapted to MRC–5 cells. The cytopathogenic effect (CPE) at the first passage was observed only in cultures seeded with Leningrade-16 (at 4 days), most probably because this strain has been previously adapted to WI–38 HDC (Andzaparidze, Darofeev, Dosser, Unanov & Rapoport, 1968). At the 2nd and 3rd passage, however, small giant cells were observed at 7–9 days post-inoculation for the other viruses and by the 4th passage the time for the appearance of CPE for the different viruses ranged between 5 and 7 days. The 5th passage therefore was made in monolayers of MRC–5 cells in Roux bottles of 1 l and for each virus 20 Roux bottles were seeded with a multiplicity of 0.01 TCID50/cell. All cutures were incubated at 33°C, except those of Sugiyama 5F100 which were incubated at 30°C. The maintenance medium which was Parker 199, supplemented with 0.2% gelatin at pH 7.6, was changed 5 days after infection at the time the first CPE was observed. Two days later the fluid was harvested and fresh maintenance medium was added after which time the viurs suspensions were harvested daily and several harvests were made. The harvests for each virus showing a titer of 4.0 log 10 or more were pooled to represent a single batch of virus and they were stored frozen at – 70°C. For the preparation of experimnetal vaccines, the frozen suspensions of each batch were thawed and the infective titers, which ranged between 4.7 and 6.2 log 10, were adjusted to 4.3 log 10 per human dose. The vaccines were blended with a stabilizer, distributed into vials of 2.5 ml (5 doses) and were lyophilized. The titer dropped after lyophilization to between 3.9 and 4.2 log 10 per human dose.

Neurovirulence in baby hamster

The results of neurovirulence tests are shown in Table 2; in addition to attenuated strains used in this study, other attenuated and virulent strains were included in the neurovirulence tests. Sugiyama BK82 was isolated directly in

TABLE 1: Karyological data on HDC, MRC-5

Lot no.	Passage no.	Hyperdiploidy (%)	Hypodiploidy (%)	Polyploidy (%)	Breaks and gaps (%)	Structural abnormalities (%)
54–4	28	0	5	6	3	0
54–5	28	0	6	5	5	1
54–4	29	0	3	4	4	0
54–5	29	0	2	3	3	0
54–4	30	0	2	3	5	1
54–5	30	1	9	4	4	0
54–4	31	0	7	4	4	0
54–5	31	0	5	5	3	0
Mean		0·12%	4·8%	4·2%	4·5%	0·25%

HDC from batch no. 6 of our local production; Beckenham 31 was isolated from a batch of commercial vaccine as described before (Mirchamsy, Razavi

TABLE 2. Neurovirulence of different strains of measles virus for baby hamster

Virus strains		Titer ($TCID_{50}$/brain) in different passages in diploid cells				
		0*	5*	10*	15*	20*
Attenuated vaccine strains	Sugiyama (5F100)	0·0	4·45	5·9	4·65	4·15
	AIK-C	4·45	5·45	4·15	5·15	4·75
	Schwarz	3·9	4·07	4·65	5·4	6·15
	Biken-CAM	3·4	5·0	4·4	Not done	6·0
	Leningrade-16	4·9	6·0	4·9	Not done	6·4
	Beckenham 31	4·4	4·9	4·77	5·9	4·9
	Biken-CAM 70	6·5	6·9	6·4	6·9	7·0
	Sugiyama BK82	0·0	5·45	6·15	5·15	5·65
	Edmonston–Zagreb	5·65	Not done	Not done	Not done	Not done
Virulent strains	Edmonston virulent	4·45	4·67	5·4	4·37	6·4
	TANABE	4·9	6·15	5·65	5·4	5·9

*Number of passages of virus in MRC-5 cells.

& Ahourai, 1972); Biken-CAM 70 virus was kindly supplied by Professor Okuno and the Tanabe virulent measles virus was received from Dr Hashizume of the Chiba Serum Institute, Japan. These strains were passed 20 times in MRC–5 cells for the study of the changes in their neurovirulence for baby hamsters during their passage in HDC. In a preliminary investigation (unpublished data) the growth curves of Schwarz and Edmonston virulent measles viruses were studied in 1-day-old hamsters. It was found that when an inoculum of 0.02 ml of these viruses (titer about 5 log 10 or more) was inoculated intracerebrally into day-old hamsters and the brains from some of the animals of each group harvested daily, the new virus cannot be detected in the brains 24 h after inoculation, but the titer of the virus increases gradually from the second day, reaching a maximum in 7 days. This is in accordance with the findings of Shishido, Katow, Kobune & Sato (1973) using the Tycsa and Leningrade-16 strains of measles virus. In the present study, therefore, the brains were harvested 7 days after inoculation when the virus titer was supposed to be at a maximum. It is intersting

TABLE 3. Results of determination of the rct40 marker

Virus strain	Passages at ° C	No. of passages at the given temperature	Log TCD_{50}/ml (mean values) 35 °C	40 °C	Log I 40*	Index rct40†	rct40 character‡
Biken-CAM	33	0	5·5	4·75	0·75	1	—
		5	4·85	2·27	2·58	3·44	—
Leningrade–16	33	0	4·7	3·65	1·05	1	—
		5	5·7	4·45	1·25	1·19	—
Schwarz	33	0	3·35	2·05	1·3	1	—
		5	4·3	1·2	3·1	2·38	—
AIK-C	33	0	4·95	1·25	3·55	0·95	—
		5	4·8	1·25	3·55	0·95	—
Sugiyama 5F100	30	0	4·75	1·25	3·5	1	—
		5	4·55	1·25	3·3	0·94	—

* Log I 40 = index of inhibition at 40 °C, i.e. the difference between log TCD_{50} at 35 and 40 °C.
† Index rct40 = ratio of log I 40 of a given strain passed 5 times in MRC-5 cells to that of its original virus.
‡ Character rct40 was determined on the basis of indices as follows: $>0{\cdot}75$ = rct40−, $0{\cdot}75$–$0{\cdot}33$ = rct40±, $<0{\cdot}3$ = rct40+.

TABLE 4. Results of determination of T50 marker

Virus strain	Passage at °C	No. of passages at the given temperature	Log TCD_{50}/ml (mean values) 50 °C 15 min	Log TCD_{50}/ml (mean values) Without treatment	Log I 50	Index T50	Character T50
Biken-CAM	33	0	4·37	5·75	1·38	1·0	—
		5	2·5	4·0	1·5	1·09	—
		10	3·5	4·75	1·25	0·90	—
		15	2·5	4·92	2·42	1·75	—
		20	2·25	4·75	2·50	1·81	—
Leningrade–16	33	0	4·5	5·37	0·87	1·0	—
		5	4·62	5·87	1·25	1·43	—
		10	4·87	6·5	1·63	1·87	—
		15	3·75	5·75	2·0	2·29	—
		20	5·0	6·0	1·0	1·14	—
Schwarz	33	0	1·25	5·25	4·0	1·0	—
		5	1·62	4·75	3·13	0·78	—
		10	1·12	4·5	3·38	0·84	—
		15	1·25	4·62	3·37	0·84	—
		20	2·5	5·75	3·25	0·81	—
AIK-C	33	0	2·62	5·12	2·5	1·0	—
		5	1·87	6·12	3·25	1·3	—
		10	4·25	6·5	2·25	0·9	—
		15	3·12	5·0	1·87	0·75	—
		20	3·5	5·5	2·0	0·8	—
Sugiyama 5F100	30	0	1·62	4·0	2·38	1·0	—
		5	2·5	4·5	2·0	0·83	—
		10	3·12	5·0	1·87	0·78	—
		15	1·5	4·87	3·37	1·42	—
		20	3·12	5·0	1·87	0 78	—

to note that all attenuated and virulent viruses of all origins both before and after adaptation to HDC were virulent for baby hamsters and the variation of neurovirulence during the passages in HDC was not significant. The Sugiyama attenuated strain BK 82 and its new further attenuated derivative 5F100, which in this study and in previous investigations were found to be non-virulent for baby hamsters, gained virulence for day-old hamsters after 5 passages in HDC, when compared with the other strains.

RCT40

The results of the rct40 marker test are shown in Table 3 in which the rct40 values after 5 passages in MRC–5 cells is compared with that of the original virus before adaptation to HDC. It is clear that the rct40 marker was not changed in 5 serial passages in MDC.

T50

The results of T50 tests are shown in Table 4. The T50 of all viruses did not change by passages for 5–20 times in MRC–5 cells.

S Marker

The plaque size variation was identical for the Schwarz, Leningrade-16 and Sugiyama 5 F100 strains which were S– at 36°C and S at 33°C both originally and after 5 passages in MRC–5 cells. This character was changed into S+, however, when the viruses were passed 20 times in MRC–5 cells and plaqued at 33°C. For the AIK-C and Biken-CAM strains both originally and after 5 or 20 passages in MRC-5 cells the plaques were S– at 36°C but there was a change of S– to S± when the Biken-CAM virus was passaged 20 times in HDC and plaqued at 33°C.

Amongst the control viruses the Edmonston–Zagreb attenuated virus showed plaques of S± and S+ at 36 and 33°C respectively whereas the Edmonston virulent virus, not adapted to HDC or adapted by 5 serial passages in MRC–5 cells, showed plaques with a S± character both at 33 and 36°C. When the number of passages in MRC–5 cells reached 20, however, the plaques remained S± at 33°C but became S+ at 36°C.

Clinical responses

Clinical data are reported for 839 children who were initially seronegative and who produced measles HI antibody after vaccination. The distribution of the children into each of the six groups by sex and age is shown in Table 5. The average onset of fever differed from 7–9 days for Schwarz, Leningrade-16, AIK-C and Biken-CAM vaccines and up to 11 days for Sugiyama 5F100 vaccine and the incidence of a fever of 37.5°C varied amongst the children

from 25% for AIK-C to 70% for Sugiyama. The mean maximum temperature,

TABLE 5. Age and sex distribution of inoculated children

Type of Vaccine	Total inoculated	Sex		Age (years)					
		F	M	1	2	3	4	5	6
AIK-C	133	67	66	19	19	28	29	19	19
Biken-CAM	140	62	78	17	22	31	24	24	22
Sugiyama 5F100	158	81	77	10	30	40	32	21	25
Leningrade-16	163	78	85	25	26	30	25	29	28
Schwarz	178	87	91	30	28	28	30	32	30
Rouvax	67	37	30	12	12	12	13	9	9
Total	839								

with the exception of AIK-C vaccine (which was 37.8°C), was between 38.5 and 38.8°C (see Table 6) and the mean duration of pyrexia (1.6–2.4 days) was not significantly different for all the vaccinees. Rashes were generally mild consisting of sporadic and discrete elements although a few more generalized rashes were seen for all except the AIK-C vaccine. The percentage of children showing a rash in the six groups was not significantly different but more severe rashes were seen in the children given Sugiyama 5F100 vaccine. The percentage of upper respiratory tract reactions such as cough, coryza or conjunctivitis was low and Koplic spots were observed in 1–2% of vaccinees except for those immunized with the AIK-C vaccine. No convulsion was reported during the field trial.

The serological responses (see Table 7) show that AIK-C and Biken-CAM vaccines which caused less reactions gave the lowest titers. This is in agreement with the observation of Cockburn, Pecenka & Surdaresan (1966), who compared four live attenuated measles vaccines and found that the antibody levels were the lowest with the vaccines causing the least reaction and highest with the vaccines giving rise to more reactions. The antibody responses are shown also diagrammatically in Figure 2 from which it can be seen that the cumulatine titer distribution curves for the five vaccines other than Biken-CAM are almost parallel. The mean antibody titers, however, were different ranging between $2^{5\cdot2}$ for AIK-C vaccine and $2^{6\cdot5}$ for Schwarz vaccine.

DISCUSSION

Adaptation of several attenuated strains of measles viurs by growth in MRC-5 HDC was rapid and it was found that by using a low inoculum of about 1 infective particle per 10–100 cells, a high titer of viurs was obtained. These findings are similar to those of Ikic, Lulic, Dedic & Asaj (1968), who passaged the Edmonston – Zagreb strain in WI-38 HDC. The measles vaccine produced in HDC, compared with the vaccine prepared in SPF-chicken cell, is very eco-

TABLE 6. Febrile reaction and clinical symptoms in serum negative and seroconverted children

Type of vaccine	No. observed	Fever			Rash	Koplik	Respiratory symptoms	Convulsion
		> 37·5 °C	Mean maximum temperature (° C)	Mean duration (days)				
AIK-C	133	33 (25·1)	37·8	1·6	34 (25)	0 (0)	13 (9·7)	0
Biken-CAM	140	77 (55·1)	38·5	2·1	35 (25)	2 (1·4)	8 (5·7)	0
Sugiyama 5F100	158	112 (70)	38·6	2·4	50 (31)	4 (2·5)	30 (18)	0
Leningrade-16	163	90 (55)	38·8	2·3	60 (36)	2 (1·2)	29 (17)	0
Schwarz	178	92 (52)	38·7	2·4	53 (29)	3 (1·6)	27 (15)	0
Control Rouvax	67	30 (44)	38·5	2·2	17 (25)	1 (1·4)	12 (18)	0

Figures in brackets represent percentages.

TABLE 7. Measles antibody responses of initially seronegative children 4 weeks after inoculation of different live attenuated measles vaccines produced in MRC-5 diploid cell

Type of vaccine	No. tested	Natural antibodies	HI-antibody titer (log 2n)									Take rate (%)	Antibody titer (g)
			Negative	2·0 3·0	3·5 4·0	4·5 5·0	5·5 6·0	6·5 7·0	7·5 8·0	8·5 9·0	9·5 10·0		
AIK-C	171	38	0*	2	12	26	55	26	6	1	5	100	$2^{5\cdot2}$
			0†	(1·7)	(9·1)	19·6	(41.5)	(19·6)	(4·6)	(0·7)	(3·2)		
Biken-CAM	198	58	6	0	7	47	48	27	5	0	0	95·5	$2^{5\cdot6}$
			(4·5)	(0)	(50)	(33·5)	(34·3)	(19·2)	(3·5)	(0)	(0)		
Sugiyama 5F100	173	15	7	2	11	51	34	33	11	5	4	95·6	$2^{5\cdot7}$
			(4·4)	(1·3)	(7·0)	(32·3)	(21·5)	(20·8)	(7·0)	(3·2)	(2·5)		
Leningrade-16	212	49	0	0	0	17	47	62	32	5	0	100	$2^{6\cdot4}$
			(0)	(0)	(0)	(10·1)	(29·0)	(38·0)	(19·8)	(3·1)	(0)		
Schwarz	232	54	6	1	17	8	36	62	33	11	4	96·7	$2^{6\cdot5}$
			(3·3)	(0·5)	(9·6)	(4·6)	(20·3)	(34·8)	(18·6)	(6·1)	(2·2)		
Control Rouvax–Schwarz	71	4	3	3	2	6	20	21	9	2	0	95·5	$2^{6\cdot0}$
			(4·5)	(6·0)	(3·0)	(9·0)	(29·8)	(31·3)	(13·4)	(3·0)	(0)		

* Number of cases.
† Per cent (seroconversion rate).

nomic because it is possible to obtain daily harvests and from a single Roux bottle of 1 l one may obtain up to 800 ml of a virus suspension with a titer of

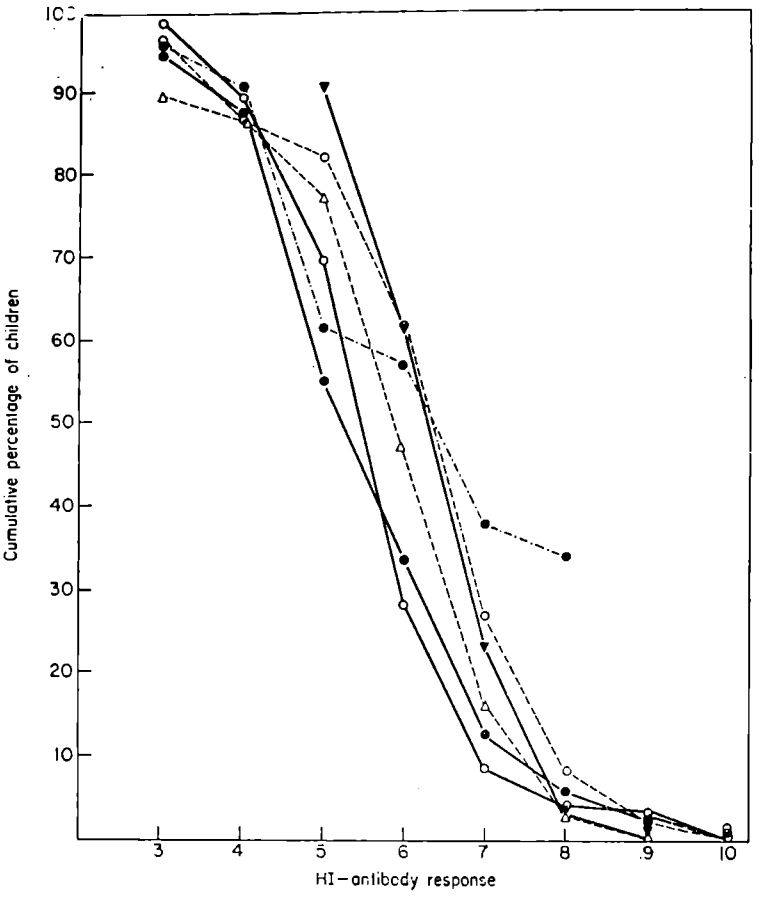

Fig. 2. HI-antibody responses in vaccinees initially seronegative. AIK-C (○——○); Biken-CAM (●—·—·—●); Sugiyama 5F100 (●——●); Leningrade-16 (▼——▼); Schwarz (○- - -○); Rouvax-Schwarz (control) (△- - -△).

4.5–6.0 log 10. For technical reasons, however, the number of harvests was limited to four with the suspension of virus being harvested every other day. The change of plaque size from S– to S+ for the Schwarz, Leningrade-16 and Sugiyama 5F100 strains after 20 passages in HDC is in agreement with the finding of Khozinsky, Seibil, Panteleyeva & Shapovalova (1967), who found such a change with the Schwarz strain adapted to HDC. The preservation of the S–character during growth in HDC indicated that the AIK-C strain had a stable character and was a homogenous virus population in this respect. Our findings of an average size of 0.61mm for the plaques of the Edmonston-Zagreb strain adapted to WI-38 cells were in agreement with those of Ikic, Juzbasic,Hrabar & Cimbur-

Schreiber (1972) and, furthermore, the Edmonston virulent strain with an S+ marker originally became S– at 36°C after 20 passages in MRC–5 cells, but no changes occurred, however, when the plaquing was performed at 33°C. From these studies, therefore, it is not possible to show any differences between the attenuated and virulent strains based on their plaque sizes at different temperatures. The same conclusion has been drawn by Takaku, Sasada & Konobe (1970), who compared various strains of measles virus by plaquing in BSC-1 cells.

It is worth mentioning that in plaquing several attenuated and virulent strains of measles in Vero cells, we mainly obtained circular plaques of various sizes. The elongated plaques observed by Rapp (1964) when using the BSC-1 cell line were rarely encountered.

All vaccine strains so far studied adapted well in MRC-5 cells and showed a high capability of multiplying in the brains of day-old hamsters. The Sugiyama attenuated strain, or its new derivative (the 5F100 clone), had no virulence for baby hamsters, because the high neurovirulence for hamsters after five passages in HDC may be due either to the new host cell membrane coating the viurs or to an increase in multiplication of the more virulent-particles. The development of neurotropism in measles viruses during frequent passages in a cell system has been shown also by Matumoto, Saburi, Aoyama & Mutai (1964). The lack of analogy between rodent encephalitis and human infection is the main argument used by those who do not believe that measles virus, highly virulent for baby hamsters may be dangerous for man. Even if this concept was true, it is possible that the baby hamster is a good model for a better approach to the problems of measles encephalitis including subacute sclerosing panencephalitis (SSPE). Based on clinical data it seems that there are differences among the vaccines studied but since the follow-up in the clinical survey was not well organized, we were not able to judge the safety of any of the vaccines in this study by clinical findings alone. We can assume, however, that the AIK-C vaccine was the mildest and that the Sugiyama 5F100 vaccine gave the most severe reactions.

Seroconversion of the vaccinees, which ranged between 95.5 and 100%, was satisfactory but there were differences in the HI titers. One month after vaccination the Leningrade-16 and Schwarz vaccines gave a higher concentration of HI antibody than did the AIK-C or Biken-CAM vaccines. This difference may play a minor role in the maintenance of long-lasting immunity, since it is generally accepted that regardless of the original titer the HI or SN antibody concentration in the circulating blood is reduced gradually to a low level though it persists throughout life. The microtiter method of measuring measles HI antibody is used universally because of its simplicity but it is not a very accurate technique. According to Ueda (1971), when the HI microtiter is 5 (log 2) the neutralizing antibody titer is about 7–8 (log 2).

A low percentage of febrile reactions and rash observed following im-

munization with Sugiyama 5F100 vaccine produced in MRC-5 HDC does not detract from the use of the vaccine and it has been used largely in our country. The Sugiyama attenuated measles vaccine has been proved by the Japan Measles Vaccine Research Commission (Shishido, 1969) to be very effective and safe. Between 1968 and 1973 about six million doses of this vaccine produced in baby calf kidney cells (CK) were used in children in the rural regions of Iran without any untoward reaction (Mirchamsy, Manteghi & Saleh, 1973). The new 5F100 strain was also found to be mild and less reactogenic than its parent strain (Mirchamsy, Shafyi, Rafyi, Bahrami, Nazari & Fatemi, 1974). Over two million doses of vaccine prepared from this strain in CK have also been used in Iran. It is, however, a fact that the percentage of clinical reactions was sometimes relatively high and some physicians were reluctant to continue to use this vaccine as a prophylactic in their private clinics. Similar observations have been published by Kawana, Kaneko & Wako (1970). In agreement with Makino *et al.* (1974), who developed the AIK-C vaccine in SPF-chicken fibroblast, the AIK-C vaccine produced in MRC-5 cells seemed to be an ideal vaccine. The physicians who administered this vaccine and who have long experience also with the Schwarz, Sugiyama and Edmonston–Zagreb vaccines felt that the AIK-C vaccine was the least reactogenic measles prophylactic used so far in Iran. The seroconversion rate of 100% given by this vaccine was another advantage attributable to its efficacy. Although it was difficult to compare the clinical reactions provoked by the different vaccines as recorded by different observers, a direct relation between the reactions and serological responses has been reported in this study. The antibody levels were the lowest with the AIK-C and Biken-CAM which caused the last reactions. The relation between the antibody titer and the duration from infection will require several years of follow-up studies before a conclusion can be reached.

Acknowledgements

We thank Dr S. Hashizume of the Chiba Serum Institute and Professor Y. Okuno of the Research Institute for Microbial Diseases, Osaka University, Japan, for providing the measles vaccine strains. We thank Dr F.T. Perkins, Chief of Biological Standardization, W.H.O., Geneva (former Head of Viral Products of the National Institute for Biological Standards and Control (NIBSC), Hampstead) and Mr J.P. Jacobs, also of the NIBSC, for providing the WI-38 and MRC-5 cells. We are especially pleased to thank Dr S. Makino, of the Kitasato Institute, for giving us the measles vaccine strain AIK-C. We are indebted to Dr M. Kaveh, Director General of the Razi Institute, for his continuous support and to Dr Moussavi, Director General of the Health Division, Mazanderan, Caspian Sea Region, and his colleagues for their help in our field studies.

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