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USE OF HUMAN DIPLOID CELL, MRC-5 FOR PRODUCTION OF MEASLES AND RUBELLA VIRUS VACCINES (*)

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ABSTRACT

A further attenuated strain of measles virus, called AIK strain, developed by Makino and his colleagues at the Kitasato Institute, Tokyo, was adapted to human diploid cells (HDC), MRC-5. In a field trial it was found that this strain initiates a low rate of clinical reactions and 100% seroconversion in vaccinees.

An attenuated strain of rubella virus, called Takahashi strain, isolated by Shishido at the National Institute of Health, Japan, was also adapted to HDC, MRC-5. Brief details of production of an experimental rubella vaccine are given. Up to 20 harvests of virus were made from each culture without any apparent alteration of diploid cells.

MEASLE SVACCINE

Since the development of the live attenuated measles vaccine by Enders and his colleagues (1960), Iran was among those countries which launched mass vaccination campaigns against measles. What led to this decision was the high mortality due to measles or its effects in rural parts of the country. Our laboratory being the only center in charge of production of measles vaccine had difficulties in obtaining special pathogen-free (SPF) eggs and even when eggs were imported, the cost per dose was prohibitive. It was then decided to start production of the

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vaccine by using baby calf kidney cells as cell substrate and as strain a Japanese attenuated measles virus developed by Matumoto et al. (1962) and kindly supplied by Chiba Serum Institute, Japan. By large-scale application of this vaccine, the epidemic patterns of the disease were deeply altered and this major pediatric problem was reduced dramatically (5). It is, however, a fact that the percentage of clinical reactions following inoculation of this vaccine were sometimes high and most pediatricians were reluctant to continue to use this vaccine in their private clinics. For this reason it was decided in 1974 to use another Japanese attenuated strain of measles virus, called Aik-C, developed by S. Makino et al. (1974) and kindly supplied by Kitasato Institute, Tokyo. The seed virus received from Japan was in its 7th passage in chick embryo cells. This strain and several other known measles vaccine strains were adapted to human diploid cells (HDC), MRC-5.

We chose MRC-5 because to our knowledge it is the only suitable candidate to replace WI-38 which has been largely used for its safety and absence of adventitious agents. It is also susceptible to various viruses.

A comparative field trial was then arranged in which five differenc measles vaccine strains were used and it was found that the seroconversion was 100% for Aik vaccine; the lowest clinical reactions were also recorded for this vaccine (6).

The vaccine is now produced on a large-scale in our laboratory. The following describes our production methods.

Preparation of vaccine

The seed virus was easily adapted to MRC-5 cells. After five passages a seed lot was prepared.

MRC-5 cells between the 28th to 30th population doublings were used. The cells were grown in roller bottles of 1400 cm2. To make roller bottles, pairs of Roux bottles of cells at population doubling 26 or 28 were combined to seed one roller and one Roux bottle. In this way two Roux bottles yield 400 cm2 and will seed 1600 cm2 at a 1 to 4 cell split. Cells were grown in BME (Grand Island Biological Co., N.Y.) supplemented with X1 amino acids, vitamins, glutamin, 5% local calf serum, 100 γg/ml of kanamycin and 100 γg/ml of neomycin. A confluent sheet was observed in roller bottles 4-5 days after incubation at 36°C.

Karyological studies were given in a previous report (6). The karyological findings of MRC-5 (2) were well within the recommended minimal requirements for karyological abnormalities, as outlined by the Cell Culture Committee 1974 (8).

The Aik-C virus grew rapidly in MRC-5 with characteristic cytopathology and virus titers which were 0.5 to 1 log higher when compared with the same system in stationary Roux bottles. The virus growth medium was the same used for growth of MRC-5 cells, but it was free of serum. 0.2% gelatin, 50 γg/ml of kanamycin and 50 γ g/ml of neomycin were added. Peak titers were observed after 4-5 days but repeated harvests at 24 h intervals were possible (Table 1). On most occasions the rollers provided four harvests up to 9 days. Single harvests were pooled and after removal of cell debris by cold centrifugation were subjected to conventional tests for sterility, safety and potency.

Genetic stability of virus grown in MRC-5

Reversion to virulence constitutes a crucial problem which is the main concern for virus vaccines previously attenuated through non-primate passage. Warren (1963) has studied the stability of attenuated measles virus adapted to HDC WI-38. We have shown that (a) rct 40 marker value after 5 passages in MRC-5c ells is similar to that of the original virus before adaptation to HDC; (b) T50 marker of all attenuated measles viruses did not change by passages for 5 to 20 times in MRC-5 cells (6).

Clinical observations

The Aik-C Vaccine developed in MRC-5 cells was the subject of two separate field trials in Iran. The vaccine showed clinical evidence of hyperattenuation and was highly immunogenic (7).

Table I. Infective titers of serial harvests of measles virus, Aik-C strain, in HDC-MRC-5 in roller bottles

No of Experiment	Harvest No	Days	TCID 50/ml (Log)
1	1	5	5 0
	2	6	5 0
	3	7	5 2
	4	8	5 5
2	1	5	5 0
	2	6	5 2
	3	7	5 5
	4	8	5 67
3	1	5	5 5
	2	6	5 67
4	1	5	4 5
	2	6	5 5
5	1	5	4 5
	2	6	4 5
	3	7	4 5
6	1	5	4 5
	2	6	5 5
	3	7	4 67
7	1	5	4 67
	2	6	4 5
	3	7	5 5
	4	8	4 5
8	1	5	4 67
	2	6	5 67
	3	7	5 32
	4	8	5 23

Takahashi strain of rubella virus, isolated in 1968 by Dr Shishido of National Institute of Health, Tokyo, was used. After isolation this virus was passed six times in primary green monkey kidney cell and had a titer of $10^{4.7}$ TCID50 /ml in rabbit kidney (RK13) cell line. The virus was attenuated in our laboratory by passages at different temperatures and in a suitable cell system. Details of its attenuation will be given in another report.

Titration of rubella virus

The titration of rubella viriu was done by an interfering titration method described by Parkman et al. (1962) in rabbit kidney cell line (RK13) kindly supplied by Dr Parkman. Five tubes of this cell were used for each dilution. Five days after virus inoculation, maintenance medium was changed and two days later, when the cytopathic effect (CPE) was noticed, the first reading was made. At the same time cultures were challenged with 100TCID50/0.1 ml of vesicular stomatitis virus, Indiana serotype. 36 h later CPE of the challenged virus was also recorded and the end point interfering titer was calculated.

Table II. Serial harvests of rubella, Takahashi attenuated strain, in HDC-MRC-5 (30 Roux bottles)

Harvest No	Days post inoculation	First experiment		Second experiment	
		TC in D_{50}/ml	Volume of harvest (ml)	TC in D_{50}/ml	Volume of harvest/ml
1	11	4.5	2,500	4.75	2,900
2	13	4.25	2,750	4.75	2,870
3	17	3.75	2,850	4.50	2,750
4	19	5.0	2,850	5.0	2,800
5	22	4.0	2,750	5.0	2,730
6	25	5.0	2,800	5.5	2,840
7	27	5.25	2,830	4.75	2,800
8	30	5.0	2,720	5.0	2,770
9	32	4.5	2,700	4.5	2,820
10	34	4.75	2,650	4.0	2,850
11	36	T.V.	27,700	4.75	2,780
12	41			4.75	2,820
13	43			4.75	2,730
14	45			4.75	2,730
15	47			5.25	2,750
16	49			4.75	2,700
17	51			5.25	2,670
18	53			4.75	2,600
19	55			4.50	2,640
20	58			5.0	2,600
				T.V.	55,200

T.V. = total volume

Rubella vaccine production

Batches of 30 Roux bottles of MRC-5 cells at population doubling 28 to 30 were seeded with Takahashi attenuated strain of rubella virus. The inoculum contained 0 05 to 0.1 pfu/cell. The adsorption time was 90 min at 30°C. The virus growth medium was the same used for growth of measles Virus except that this medium contained 2% kaolin treated calf serum. The medium was replaced, 5 days later, by the same medium. The medium was finally discarded 10 days after inoculation of virus and the cells were washed twice with phosphate buffered saline (PBS) and a fresh BME medium, free of serum, but containing 0.2% gelatin and antibiotics as mentioned before, was added. Temperature of incubation was 30°C. The first harvest of virus was made on the 11th day post-inoculation and the following harvests every other day. Table II represents the number of harvests made in two experimental productions of rubella vaccine. As shown in this table, in one experiment up to 20 harvests were made in a period of 58 days after seeding cells. The repeated harvests did not alter cell sheets which microscopically looked normal. The single harvests were treated as mentioned for mealses virus.

From data presented in this report it is evident that HDC-MRC-5 is a suitable cell system for large-scale production of live attenuated measles and rubella vaccines.

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