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THE ISOLATION AND CHARACTERIZATION A HUMAN DIPLOID CELL STRAIN AND ITS USE IN PRODUCTION OF MEASLES VACCINE*

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The present work was started in September 1978 when, due to strikes, lack of dry ice or liquid nitrogen, and frequent power cuts in the Tehran area, the stocks of two standard diploid cells, WI-38 and MRC-5, were lost. At that time there was little hope of receiving new certified cells from abroad but the need for viral vaccines was increasing. Under such conditions we developed ten human diploid cell lines (HDC) from lung tissues of ten human embryos obtained from the Central Maternity Hospital of Tehranl Although we received enough MRC-5 cells

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later, through the kindness of Dr. Schild and Mr. Jacobs of the National Institute for Biological Standards and Control (Hampstead, London), the work described here was continued in order to evaluate the local HDC for their acceptability as alternative substrates for the manufacture of human virus vaccines. According to th results of our studies the life span of six out of the ten lines have clearly shown the three phases of cultural characteristics of HDC, as described by Hayflick & Moorhead [1] and Hayflick & Jacobs [2] and one line, designated R-17 , was found satisfactory for the production of measles virus. This cell strain is described here.

The culture was established from a human foetus obtained by a therapeutic abortion performed on a 19-year-old mother. The foetus was a female, 18 weeks old, , its length was 20 cm and the mother hah no physical or mental abnormalities. Both lungs of the foetus were removed aseptically eight hours after the abortion. The lungs were washed with phosphate buffered saline (PBS) and were trypsinized by classical methods.

disposable flasks of 4 oz capacity were seeded each with 25ml of a suspension of cells in growth medium.After four days of incubation at 37°C a confluent monolayer of fibroblast-like cells was formed in each flask.The cells were detached with 0.25% trypsin solution and resuspended in a medium containing BME 40%, calf serum 30% , bovine albumin 10%, sucrose 10% and DMSO 10% .In this way 1.0ml ampoules containing 2.5-3.0 X 10^6 of first passage cells per millilitre were prepared.The cells were frozen slowly and stored at -60°C . Two months later the cells were transferred to a tank containing liquid nitrogen.

For karyological study 1.0ml of frozen cell suspension was thawed in a water bath at 37°C and was seeded into a 2 oz disposable bottle with 12ml of growth medium. This culture was subcultured on a 1: 2 or 1: 4 basis as soon as a confluent monolayer was formed. Cells from this culture were examined at passages 15 to 45. For chromosome analysis a roux bottle of culture was taken . A chromosome preparation was made on the second day after subsulture according to the method of Harden & Burton. [3] In each karyology study the chromosome count was made on 100 metaphase cells. The chromatid breaks and major chromosome abnormalities were subjected to photographic reconstruction of karyotype. The polyploidy was also examined in 300 metaphases . The chromosomes of one cell were photographed for the construction of the karyotype. The karyological findings of the R-17 line (Table 1)were within the acceptable range as laid down by the WHO Expert Committee. [4]

Histocompatability (HLA) typing of six HDC lines isolated in our laboratory was performed by Dr. J.G.Bodmer of the Imperial Cancer Research Fund Laboratories (London) by the technique of Singh et al .[5] The results are shown in Table 2. This typing is of value only in identifiying the human origin of the cells. It is worth mentioning that the fibroblasts have very low levels of HLA antigens on their surfaces and foetal fibroblasts have even lower levels than adults. In the present study the reactions for the B locus were in many cases very weak and antigen assignment was possible only after repeated typing with different sets of sera.

For the control of the absence of adventitious agents

and mycoplasma, the cells were tested at each 10th passage for the presence of contaminants as specifies by WHO for the safety testing of biological products. [4] All tests for bacteria, fungi and mycoplasma and all in vitro and in vivo tests were negative.

Tests for the absence of tumorigenicity by inoculation of 10^6 cells in suckling mice treated with antithymocyte serum were negative. The cancerous KB cell line, inoculated at the same time at the same concentration, produced growing tumours in this rodent.

Table 1.Kariologial data on human diploid cell R-17.

Culture passage	Hypodi- ploidy (%)	Hyper- diploidy (%)	Poly- ploidy (%)	Breaks and gaps (%)	Structural abnormalities (%)
15-20	1	1	1	-	-
21-25	3	-	4	3	-
26-30	4	2	2	4	2
31-35	3	3	3	4	1
36-40	4	3	4	5	2*

* Two dicentrics.

Table 2.HLA antigen on 6 foetal fibroblaasts (HDC)

No.of HDC	HLA type	Gestation (months)
R-8	A1, A2, B7, B8	4
R-9	A3,AW24, BW21, B27	4
R-11	A2	3
R-12	A9 B12	4
R-14	A2,Aw23, B41	3
R-17	A9,Aw19, BW35	4.5

Five pilot lots of measles virus vaccine have been produced in cultures of MRC-5 and R-17 under conditions currently used in the manufacture of measles vaccine.Results shown in Table 3 indicate the similar susceptibility to measles virus of both cell strains.

With the cooperation of the Department of Preventive Medicine of the Ministry of Health of the Islamic Republic of Iran, children of certain areas of Semnan, a town in the north-east of the country, were selected for this study. It was known that no natural outbreak of measles had been recorded during the past 12 months in this town. Three hundred and fifty healthy children,aged ten months fo five years evenly distributed between the two sexes , without previous history of measles infection of vaccination were vaccinated. Clinical data on 120 children who were accessible in their localities were collected. The average onest of fever which occurred in 25% of children was between 7 and 9 days after vaccination . The mean maximum temperature did not exceed 38°C for a mean duration of two days. Rashes were mild and sporadic , the upper respiratory tract reactions such as coryza, conjuctivitis and cough were low,Koplik spots, otitis and convulsion were not reported in this field trial . The second blood sample of the vaccinees were collected on filter paper discs, four weeks after immunization, by a team of health workers who visited the patients house by house. According to the parents, the clinical symptoms of the vaccinated children were mild and or short duration; in many cases no reactions were noticed.

Haemagglutination-inhibition (HI) antibody titres of the children were relatively low (Table 4) with a

Table 3.Measles virus "AIK" strain susceptibility of MRC-5 and R-17

Experimental batch	MRC-5 Culture passage	MRC-5 No.of RF*	MRC-5 Mean titre**	R-17 Culture passage	R-17 No.of RF*	R-17 Mean titre
61-1	16	250	5.0	8	217	5.5
61-2	25	160	5.5	16.	225	5.2
61-3	27	230	4.85	25	336	5.5
61-4	27	140	5.5	27	276	5.82
61-5	30	185	5.7	30	312	5.5

* RF= Roux flsks seeded.

**Reciprocal $\log_{10}$ of the TCID50 per millilitre.

Table 4. Antibody response of initially seronegative children, four weeks after administration of AIK measles vaccine produced in HDC-R17.

Antibody tested	No. tested	Antibody titre ($\log_2$) Neg.*	2.0 | 3.0	3.5 | 4.0	4.5 | 5.0	5.5 | 6.0	6.5 | 7.0	7.5 | 8.0	Take rate	Antibody titre (G M)
HI**	300	12	12	26	150	78	16	6	96	$2^{4.8}$

* NEG = Negative.

** HI = Haemagglutination-inhibition.

protection coverage of 96%. The relatively low level of HI antibody observed in vaccinees , is similar to ou

previous findings in which AIK-C vaccine produced in MRC-5 cells was used. This is also in agreement with the observations of Cockburn et al[7]. Who compared four live attenuated measles vaccines and found that the antibody levels were the lowest with the vaccines causing the least reactions and highest with those causing more reactions.

There is now more evidence on the safety of HDC lines used as cell substrates for the preparation of human viral vaccines. At an early stage when HDC was increasingly used in the manufacture of human viral vaccines, there was some concern over the efficiency of existing technology in detecting potentially oncogenic agents and consequently it was suggested that a possible oncogenic agent effect of vaccines produced in HDC cannot by entirely excluded;[8] but recent investigation of Mellor et al.[9] showed that in some 3000 recipients of a vaccine developed in HDC no case of cancer was notified among the vaccinees 12 years after vaccination .

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