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A NEW FIELD TRIAL WITH TWO TYPES OF LIVE MEALSES VACCINE IN RURAL REGIONS OF IRAN(*)

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Two further attenuated measles virus vaccines, Sugiyama and Aik strains, developed in baby calf kidney cells and in Human Diploid Cells (MRC5) respectively were compared in a field trial. Both vaccines showed clinical evidence of attenuation and both were highly immunogenic.

Problems related to the success and failure of mass immunization against measles in developing countries and in hot climates are shortly reviewed.

INTRODUCTION

The local production in Iran of live attenuated measles vaccine was started early in 1970 by using Sugiyama Strain, a Japanese measles virus adapted to baby calf kidney (CK) cells by Matumoto et al (10) and developed by Chiba Serum Institute – Japan.

In two separate field trials (12,13) it was found that this vaccine is safe and highly immunogenic inducing a sero-conversion of about 95 percent in susceptible children. By the end of 1971 the number of vaccinees in our rural

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regions was more than 3.5 million, which make almost 37 percent of the susceptible age groups (9). At the end of 1972, when over 5 million children in rural areas were vaccinated with this strain at its 82 nd passage in CK cells, and progressive decrease in the incidence of measles as well as a reduction of excessive infant mortality was obtained, a further attenuated Strain of Sugiyama, called 5F 100, developed also in Chiba Serum Institute of Japan by elution of virus from AlPo4, and further passages in CK cells of the cloned virus was received and used for large scale production of live measles vaccine (16). This new strain was found to be more attenuated than the previous one. The thermal reactions in vaccinees was lower and the rash was sporadic and of short duration. Because of the high level of protection conferred by this vaccine and its low cost of production, it was largely used in the programme of mass immunization launched by Iranian ministry of Health. The only disadvantage of this vaccine was the type of morbilliform rush, similar to natural measles, observed in a low proportion of vaccinees. The incidence of fever and other clinical symptoms was however low and encephalitis due to vaccination has not so far been reported. At the end of 1975 over 7.5 million children of 9 months to 5 years in rural regions have been vaccinated.

Recently a comparative field trial was conducted in Caspian Sea area (17) by using 5 experimental vaccines manufactured with Sugiyama 5F 100, Schwarz, Leningrade 16, Biken-Cam (20) and Aik (8) strains all adapted to Human Diploid cell (HDC). The object of this work is to report another field trial in which Sugiyama 5F 100 vaccine produced in CK cells is compared with Aik virus vaccine cultured in HDC, MRC-5 cells.

Materials and Methods

Human Diploid Cell: HDC, MRC-5 cell, in a 4 oz bottle at 9th passage was kindly supplied by Dr. Jacobs of Medical Research Council, Hampstead (London). This cell was serially passaged, as suggested by Jacobs (4) the cells at the 16th passage were suspended in a medium containing 50 parts Basal Medium Eagle (BME), 20 parts calf serum, 10 parts succrose and 10 parts dimethyl Sulphoxide (DMSO). The number of cells in each milliliter of medium was 2.5×10^6. The cells were distributed 1 ml per ampule, and the ampules were flame sealed and were then gradually cooled until they reached a temperature of – 80 c and were then immersed in liquid nitrogen for storage. These cells were found to be free of mycoplasma and bacterial or viral contaminations. For production of vaccine, each time a new ampule was used. The cells were seeded either in Roux flasks or in roller bottles and were grown in BME supplemented with $1\times$ concentration of BME aminoacids, vitamins and glutamin and 10 per cent of local calf serum. Cultures were subdivided on a 1:2 or 1:4 basis as soon as the monolayer was confluent.

Karyological analysis performed at passage 29,30 and 31 is described in the previous report (17).

Aik - seed virus: Aik-C vaccine lot TV12was generously supplied by Dr.S. Makino of the Kitasato Institute - Tokyo. This is a further attenuated virus derived from Edmonston virulent strain developed by Makino et al (6,7). The virulent Edmonston strain was passed 12 times in primary sheep kidney cell at 33 c. Plaques isolated in SPF chick embryo (CE) cultures were cloned in CE cells and one clone was selected as vaccine seed virus and was called Aik-C strain. The 7th passage virus of Aik-C strain in CE cells was used for vaccine lot TV–12 (8).

Aik-C Vaccine Production: The Aik-C vaccine lot TV12 was directly subcultured (0.1 – 0.01 PFU/cell) 5 times in MRC–5 at 33 c. The cytopathic effect (CPE) consisted of the appearance of small giant cells was observed at the 2nd and 3rd passage, 7–9 days post inoculation. At the 5th passage the time for the appearance of CPE was 5–6 days. The maintenance medium was TC 199 supplemented with 0.2% gelatin, 50μg/ml neomycin and 50μg/ml kanamycin, at pH 7.6. This medium was changed 5 days after infection when the first CPE was observed. Two days later the fluid was harvested and fresh maintenance medium was added it was possible to make 3 to 5 single harvests for each batch. When confirmed, those harvests having a titer of 4.0 log or more were pooled and were aseptically passed through millipore sheets of 5 mμpore size to remove cell debris.

The virus suspension was then blended with a proper stabilizer and was distributed into vials of 2.5 ml (5 doses), and were finally lyophilized. The virus content of each batch per dose was 3.9 to 4.2 log.

Sugiyama Vaccine: This vaccine was prepared, as we have described elsewhere (16) with the cloned 5F 100 virus passed twice in CK cells in Iran (Batch No. 10). Each dose of this vaccine contains 3 log of virus after lyophilization.

Control of Vaccines: The control of safety in monkeys and in small animals and the potency tests were done by conventional methods outlined by WHO (19). The neurovirulence of viruses for day old hamsters was also determined for each batch of vaccine as described before (14).

Study Population: Children of two villages in Caspian Sea area, North of Iran and some home dwelling children at the Razi Institute and its surrounding villages have been inoculated. The vaccinees were coded by a number which was not disclosed before the end of serological survey. Children were aged 12 months to 5 years, evenly distributed between the two sexes. Vaccination was performed by trained public health technicians. Daily temperature from 7th to 15th day after immunization and significant reactions were recorded.

HI test: 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 described before (11) the serum was eluted from filter paper disks and treated with kaolin as well as with monkey red blood cells as described in previous report (12). The paired sera of each child were tested simultaneously by Hemagglutination Inhibition (HI) test according to Rosen's technique described before (12).

Pathogenesis for suckling hamster: In 3 experiments the virulence of the two strains for suckling hamsters was compared. The results are illustrated in table No 1. In this study the Aik strain was found virulent for baby hamsters while the sugiyama viruses have shown a very low virulence for this rodent.

Clinical response: The distribution of children in each of the two groups is reflected in table 2. The average onset of the fever was 12 days for sugiyama and 9 days for Aik vaccine. In both cases fever was mild about 50 percent of susceptible children showed a rise of temperature of about 1°C. for about 1 day. It seems however that the febrile reaction was milder in children inoculated with Aik vaccine (Table 3). The percentage with rash was 35 per 100 for sug. 5F 100 and 25 per cent for Aik vaccine. While the original sugiyama vaccine consistently showed a morbilliform rash, in most of susceptible children, indistinguishable from natural measles, with the sug. 5F 100 strain the number of cases and severity of rash was reduced; however in a low proportion of vaccinees rash was still severe and persistent. This was the only weak point of sugiyama strain which is not tolerated by some physicians and parents of children. With Aik vaccine the rash was always mild and consited of tiny exanthema which faded the second day after appearance. Respiratory disorders such as cough, coryza, tonsilitis etc were more severe with sug. 5F 100 vaccine than with Aik (table 4); koplik spots and convulsion were not observed for neither vaccines.

The seroconversion rate was 94.6 and 97.8 for sug. 5F 100 and Aik vaccine respectively. The mean antibody titre was about 1 log2 lower in childern immunized with Aik (table 5).

Discussion

The high mortality due to measles complications, exceeding 10.000 cases a year in Iran (18) justified a regular mass campaign against the disease in remote rural areas of the country. In these sections of the country where a high percentage of the population had before land reform unsatisfactory economic conditions, measles complications were the main cause of mortality in undernurished children. The situation was gradually improved after the social reforms and

the result of regular mass immunization against measles, launched early in 1970 with attenuated and further attenuated strains of sugiyama vaccine resulted in dramatic decrease in morbidity and mortality due to measles infection (9). In 1975 the number of reported cases was one tenth of that in 1966 when mass immunization was planned (table 6) we are still far from a good coverage of nearly all susceptible children and small outbreaks of the disease in rural areas still occur. Although there is no doubt about the effectiveness of the mealses vaccine but the failure of eradication should be attributed to the failure of widespread vaccination.

The hope of eradication of measles in urban or rural regions will be dashed if a regular mass campaign covering nearly all children 1 to 2 years old is not performed. In another word the protection of a community requires immunizing most if not all, of susceptible children. The example of the United State of America where the usual morbidity of measles was about 500.000 cases some 15 years ago, or the number of reported cases was decreased, thanks to mass vaccination, to 62.333 in 1966 and to 23.000 in 1968 is interesting to be mentioned. This low incidence has risen to more than 75.290 in 1971. In that year direct federal funds for measles vaccination was reinstituted, and the upward case trend was reversed in 1972, when 32275 cases of measles were reported (1). There is therefore a direct relation between the morbidity due to measles and the extension of mass immunization.

While measles in rural regions of Iran was decreasing, the health authorities were faced with increase of measles in the cities specially in industrial centers with dense population (15). At this stage the health and economic aspects of the problem was important and consequently the mass campaign was introduced in large cities among children attending collective institutions, kindergartens and creches. The vaccine is also available in all health centers where parents, regardless of their socio-economic conditions, can refer for free vaccination of their children. The private medical practice, on the other hand, uses the live measles vaccine when mothers attend the clinics for medical cares. As we have mentioned before, at the end of 1975 over 7.5 million children of rural areas were vaccinated with the mentioned sugiyama vaccines. The clinical symptoms due to this vaccine were not sometimes acceptable to the physicians who immunized children in their private clinics. The severity of rash, although rare, was the main reason of fear for those who believed that there is little difference between natural measles and the reaction of sugiyama live attenuated vaccine. Although this fear was groundless and it was quite normal to see the live vaccine induces, in a proportion of vaccinees, a febrile disturbance or a mild, non infecious measles like illness; however we tried to provide a more attenuated measles vaccine with much less clinical symptoms. AIK vaccine, produced in our laboratory in HDC-MRC5 was the ideal vaccine which produce the lowest clinical manifestation. The results of the present field trial assure that

the vaccine is quite safe and immunogenic. The difference in pathogenesis of Aik and sugiyama strains for day old hamster is not, at the moment, a marker of a significant factor for or against any of these strains. In a previous report it has been shown (17) that attenuated measles strain derived from Edmonston strain as well as other measles vaccine strains are virulent for day old hamster. The Sugiyama attenuated or further attenuated viruses , on the other hand, adapted to CK cells are rarely pathogenic for baby hamster but once passaged in HDC they become virulent for this rodent. The pathology of Human and Hamster not being the same, it is hard to suggest any relation between the neurovirulence of mealses vaccine strains and post vaccinal hazards.

It is also worth to mention that the Aik vaccine is an expensive prophylactic even if adminstered in mass campaigns. In accordance with other investigators (3) we have found the possibility of reducing the dosage of Sugiyama further attenuated vaccine from 1000 TCID50 per dose to 200 TCID50 per dose without significant difference in the rate of seroconversion (unpublished). This experiment hadnot been performed with AIK vaccine; on the contrary we try to lyophilize AIK vaccine containing 5000 to 10.000 TCID50 per dose in order to assure a convenient concentration of live virus when vaccine is used in warm remote areas. It is well known that the infectivity titre of a lyophilized measles vaccine will decrease with storage at room temperature, and this is accelerated after the vaccine has been rehydrated. Therefore it would be risky, to reduce the dosage of vaccine when if is adminstered in tropics for mass use. There are other factors which may also interfere with successful mass Immunization. These factors, in developing countries are numerous. Lack of good communication between villages, lack of cooperation of people with health teams in charge of vaccination, poor refrigeration of vaccines, difficulties in keeping reliable records, absence, of children during the followup studies are among many other problems which may arise when a mass campaign is performed. There are other basic factors which also may affect the results of mass immunization. The vaccination at an early age may be unsuccessful because of the interfering effect of maternal antibody. Krugman et al (5) detected HI antibody in the majority of babies at 6 months of age. In two different Surveys we have found (12,16) that 7.7. and 7 percent of children aged 9 to 12 months had maternal HI antibody and did not respond to the vaccination, to this we should add 5 to 7 per cent of children who normally do not respond to vaccination and remain susceptible to measles. In other word even if mass campaign is performed in the best possible way, about 15 percent of susceptible children would remain unprotected. This proportion tends to increase with the migration of children to and from areas with lower vaccination rate. Therefore with the present level of vaccination, the eradication of measles is unobtainable but the disease will remain a low-grade endemic infection.

Finally the short duration of measles susceptibility in warm climates is another obstacle to the implentation of effective immunization. As a matter of fact it is generally observed that in temperate countries, infants remaining measles susceptible for 2 to 4 years before they contact the disease, so nearly all are measles vaccine responsive between the age of 12 months and 3 years (Griffith, 1975) (20. In the tropics however mealses is caught much earlier in life, with the result that if children are vaccinated at the age of 9 to 12 months a significant proportion having maternal antibody will not respond to the vaccine but this group remain susceptible after a short time and will be infected, a few months later, by natural measles. People will then attribute this failure to the inefficacy of the vaccine. On the countrary if children in hot climates are not immunized until the age of 15 months, a substantial proportion of them have aready been infected by measles whilst a small proportion will still be protected by maternal antibedies. In order to avoid this difficulties it is suggested to vaccinate children at the age of 12 months and to revaccinate them six months later (2) Another suggestion is to continue programme of mass immunization. Failure to do so would rapidly result in a return to the current high prevalence of measles in young children.

TABLE 1. NEUROVIRULENCE OF THE TWO MEASLES VACCINE STRAINS FOR DAY OLD HAMSTER

No. of experiment	Vaccine strain	Log TCID 50/Brain in vero cells
1	Sugiyama 5F 100	0.20
	AIK	4.65
2	Sugiyama 5F 100	0.0
	AIK	4.35
3	Sugiyama 5F 100	0.0
	AIK	4.40

TABLE 2. AGE AND SEX DISTRIBUTION OF INOCULATED CHILDREN

Type of vaccine	Total inoculated	Sex		Age (years)		
		Female	Male	9–12 Months	1–2	2–5
Sug-5F 100	112	60	52	40	38	34
AIK	123	67	56	34	42	47

TABLE 3. FEBRILE REACTION AND CLINICAL OBSERVATION IN SERO NEGATIVE CHILDREN

Type of vaccine	No. of children	Fever (C°) 37–38	38–39	39	Onset Mean (days)	Mean duration of pyrexia (days)	Mean duration of maximum temperature (days)
Sug-5F 100	102	65(64%)	6(2%)	2(2%)	12	1.5	1.0
AIK	105	58(55%)	2(2%)	0(0%)	9	1.6	1.0

TABLE 4. INCIDENCE OF RASH AND CLINICAL OBSERVATION

Type of vaccine	No. of children	Rash (%)	Koplik spot (%)	Respiratory symptoms (%)	Convulsion (%)
Sug-5F 100	102	36 (35)	(0)	37 (53)	(0)
AIK	105	26 (25)	(0)	11 (10)	(0)

TABLE 5. SEROLOGIC RESPONSE IN INITIALLY SERONEGATIVE CHILDREN

Type of vaccine	No of sera tested	Children with maternal antibody	Seroconversion			%	GMT*
			Convertors	/	vaccinated		
Sug - 5F 100	102	9	88	/	93	94.6	6.0
AIK	105	15	88	/	90	97.7	5.1

* Geometric Mean Titre : HI titre log 2.

TABLE 6. MORBIDITY FROM MEASLES IN RURAL AREAS OF IRAN 1966–1975

Years	Total cases reported
1966	127514
1967	92752
1968	94365
1969	84486
1970	63751
1971	57545
1972	50142
1973	42994
1974	16589
1975	13873

REFERENCES

1 – Barkin, R.M. and Conrad, J.L. (1973)– Current status of measles in the United States J. Infect. Dis. 128, 353–356.

2 – Griffith, A.H. (1975)– Measles vaccination in tropical countries. Royal Sec. Trop. Med. Hyg. 69, 29–30.

3 – Hendrickse, R.G. and Montefiore, D. (1968)– Measles vaccination with reduced dosage. Brit. Med.J. 4, 28–30.

4 – Jacobs, J.P. (1968) – The production and control of a standard cell substrate for producing viral vaccines. Proc. Sympos. Oncogen. Virus vaccines. Yugoslav Acad. Sci. and Arts Zagreb, 81–92.

5 – Krugman, S., Giles, J.P. and Friedman, H. (1967) – Studies on immunity to mealses: First internatinal conference on vaccines aagainst viral and Rickettsial Diseases of Man, Scientific Publication No. 147 Pan American Health Organization PP 353–360.

6 – Makino, S. et al (1973) – Field trial with a further attenuated live measles virus vacicne. Jap. J. Microb. 17,75–79.

7 – Makino, S. et al (1973) – Evaluation of the live AIK measles vaccine. The Kitasato Archives of Exp. Med. 46, 83–92.

8 – Makino, S. et al (1974) – Studies on the modification of the live AIK measles vaccine. II. Development and evaluation of the AIK–C mealses vaccine. The Kitasato Archives of Exp. Med. 47, 13–21.

9 – Manteghi, A. (1972) – Measles and its epidemiological evalution following vaccination

in Iran (1961–1971). In Persian) Report No. 41 Ministry of Health-Iran.

10 – Matumoto, M., Mutai, M., Saburi, Y. Fuji R., Minamitani, M. and Nakamura, K. (1962) – Live measles virus vaccine: Clinical trial of vaccine prepared from a variant of the Sugiyama strain adapted to bovine kidney cells. Japanese Journal of Experimental Medicine 32, 433–448.

11 – Mirchamsy, H., Nazari, F., Stellman, C. and Esterabady, H. (1968) – The use of dried blood absorbed on filter paper for the evaluation of diphtheria and tetanus antitoxins in mass survey. Bull World Health Organ. 38, 665–671.

12 – Mirchamsy, H., Shafyi, A., Bahrami, S., Bassali, Y. and Nazari, F. (1970) – A comparative study of two live measles vaccine in Iran. J. Hyg. Camb. 68, 101–110.

13 – Mirchamsy, H. Shafyi, A., Bahrami, S., Nazari, P., Mirzadeh, M. and Bassali, Y. (1971)– Mass immunization of children in Iran with live attenuated Sugiyama measles virus adapted to calf kidney.-Jap. J. Exp. Med. 41, 39–48.

14 – Mirchamsy, H., Razavi, J. and Ahurai, P. (1972) – Pathogenesis of vaccine strains of measles virus in suckling hamsters. Acta Virol. 16, 77–79.

15 – Mirchamsy, H., Manteghi, A. and Saleh, H. (1973) – Efficacy and safety of live attenuated Sugiyama strain of measles virus in mass immunization of children in rural region of Iran. Proc. Symposium on Field trials of vaccines. Yugoslav Academy of Sci. and Art, Zagreb 123–130.

16 – Mirchamsy, H., Shafyi, A., Rafyi, M.R., Bahrami, S., Nazari, P. and Fatemie, S. (1974)– Experimental study of a further attenuated live measles-vaccine. J. Hyg. Camb. 72, 273–279.

17 – Mirchamsy, H., Shafyi, A., Bahrami, S., Kamali, M., Nazari, P. Razavi, J., Ahourai, P., Fatemie, S., and Amin Salehi. A comparative field trial of five measles vaccines pro- P., Fatemie, S , and Amin Salehi. A comparative field trial of five measles vaccines produced in Human Diploid Cell, MRC–5. Journal of Biological Standardization 1976, 5, 1 .

18 – Naficy, K., Saidi, S., Nategh, R. Mostatab, A. and Akbarshahy, R. (1967)– Comparative study of live attenuated measles vaccines in rural areas of ran. Arch. ges. Virusfor. 22, 11–22.

19 – Requirements for measles vaccine (live) and measles vaccine (inactivated). World Health Organization, Technical Report series, 1966 No. 329.

20 – Takaku, K. et al (1970) – Studies on further attenuated live meas es vaccines III. Selection of less reactogenic variants of CAM measles vaccine virus. Biken J. 13, 163–168.