J. Hyg., Camb. (1974), **72**, 273
Printed in Great Britain

Experimental study of a further attenuated live measles vaccine of the Sugiyama strain in Iran

By H. MIRCHAMSY, A. SHAFYI, M. R. RAFYI, S. BAHRAMI, P. NAZARI and S. FATEMIE
State Razi Institute, P.O. Box 656-Tehran, Iran

(*Received* 7 *August* 1973)

SUMMARY

After encouraging results of the mass vaccination programme in Iran, in which 5 million children in rural areas were vaccinated with the Japanese Sugiyama strain at its 82nd passage in baby calf kidney, and a progressive decrease in the incidence of measles as well as a reduction of excessive infant mortality, a further attenuated vaccine, produced with the same strain, cloned in Japan, was compared in a field trial with the parent vaccine. The new strain caused fewer reactions than the original strain. Seroconversion with a geometric mean antibody titre of 6·1 was observed in 95% of susceptible children.

INTRODUCTION

The live attenuated measles viruses now in use for measles prevention are normally propagated either in leukosis-free chick embryo cells or in primary monkey kidney cells. There are, however, strains such as Sugiyama attenuated virus adapted to calf kidney (CK) (Matumoto *et al.* 1962) or ESC and AIK strains both developed in lamb kidney cells (Chumakov *et al.* 1967; Makino *et al.* 1973). Each cell system may have several advantages as well as some shortcomings. In Iran, since 1968, the Sugiyama strain adapted to CK cells has been largely used in all rural regions where measles was the main problem. The mortality due to measles complications in those regions, estimated to be at least 10,000 per year before the mass campaign, has declined dramatically after 5 years of extensive immunization against the disease. The choice of the attenuated Sugiyama strain of measles virus was based on the following points:

(*a*) The virus is easily propagated in primary baby CK cells. Because of the repeated harvests of virus, once grown in this cell system, the production of large amount of virus at a low cost, to cover the needs of mass immunization, was possible.

(*b*) Baby CK cell is normally free of the well-known contaminants of eggs or monkey cells. Unlike ovine kidney cells which frequently show slow-growing agents with cytopathogenic effect, we have not so far observed any cytopathogenic effect of unknown origin in CK cells. Since 1968 over six million doses of the vaccine have been produced in baby CK cells at passage 82. The vaccine was well tolerated by children of 1–5 years who have shown a seroconversion rate of about 95%,

4 weeks after vaccination. The Sugiyama attenuated strain was first developed in Japan by Matumoto *et al.* (1962) and has been investigated by Myamura *et al.* (1971). According to the latter authors this strain has gradually been attenuated by passage in CK cells at low temperature, but the febrile reaction of vaccinees had not been decreased from the 70th to 86th passage. The virus from the 81st passage in CK cells was recently cloned by Hashizume and his colleagues after elution of virus from $AlPO_4$. Two batches of vaccine were prepared by these authors with the cloned virus, called 5F, after 4 or 19 further passages of the clone in CK cells. Thus the vaccines were prepared at passage 85 or 100 of the Sugiyama strain in CK cells. The vaccines were studied by these authors in a field trial with the following results:

(1) No difference was observed between the two viruses regarding neurovirulence in the monkey, *in vitro* markers, or production of reactions in susceptible children.

(2) The cloned virus did not grow at 39·2° C., at which temperature the original virus grew slightly.

(3) Febrile reactions of vaccinees inoculated with the two vaccines were decreased when compared with the parent vaccine.

(4) After inoculation of these vaccines, the rash was mostly scanty, consisting of tiny spots. The rash from the original virus was mostly maculo-papular or urticarial.

After this field trial, Dr Hashizume, Chief of Measles Unit, Chiba Serum Institute, Japan, generously supplied us with enough seed material of 5F clone to start production of our own seed lot.

The object of the present study was to compare the 5F cloned virus with the original Sugiyama strain at passage 82 in CK cells which has so far been largely used in Iran.

METHODS AND MATERIALS

Vaccines used

The Sugiyama virus at its 82nd passage in CK cells was part of a large batch of vaccine used in the country since June 1971. Each single dose of vaccine contained $10^{2·7}$ TCID 50 of virus. The further attenuated vaccine was prepared with the cloned 5F passed twice in CK cells. One dose of this vaccine contained $10^{3·0}$ TCID 50 of virus. Both vaccines were lyophilized and stored at $-20°$ C. before use.

Pathogensis for suckling hamster

In order to compare the virulence of the two strains for suckling hamsters, litters of five to seven baby hamsters 1–2 days old were injected intracerbrally with 0·02 ml. of undiluted virus, immediately after reconstitution of the lyophilized vaccine. Signs of illness and procedure for isolation of virus have been given before (Mirchamsy, Razavi & Ahourai, 1972).

Table 1. *Age and sex distribution of inoculated children*

Type of vaccine	Total inoculated	Sex		Age (years)		
		Female	Male	10–12 months	> 1–2	> 2–5
Sug. 82	118	54	64	22	44	52
5F 100	75	40	35	27	27	21

Table 2. *Number of children developing pyrexia*

Type of vaccine	No. of children	Fever (°C.)			Onset mean (days)	Mean duration of pyrexia (days)	Mean duration of maximum temperature (days)
		37–38	38–39	> 39			
Sug. 82	85	46 (54%)	16 (19%)	3 (3·5%)	8·11	4·22	2·6
5F 100	70	45 (64%)	4 (5·7%)	1 (1·4%)	12	1·5	1·0

Children

The study was carried out on 193 home-dwelling children in the Razi Institute and its surrounding villages. The children had not been immunized before against the disease and were without past history of clinical measles. The children were allocated to one of the two groups by the random sampling method.

Clinical observations

The temperature was recorded twice daily for 3 weeks after vaccination. The clinical reactions were recorded by trained technicians who paid regular visits to the children. Mothers were also advised to refer to the clinic of the Razi Institute when clinical manifestations were noticed. During the febrile period, each child was examined on 5 consecutive days by a physician either at home or in the clinic.

Serological testing

In order to evaluate the antibody response to vaccination, blood samples were collected immediately before inoculation and 30 days later. The blood was collected from finger pricks using paper disks as described previously (Mirchamsy, Nazari, Stellman & Esterabady, 1968). The paired sera of each child were tested simultaneously by the hemagglutination inhibition (HI) test according to Rosen's technique (Mirchamsy *et al.* 1970).

RESULTS

Clinical response

The distribution of children in each of the two groups according to sex and age is shown in Table 1. The average onset of the fever was 8–11 days for the original vaccine. This period was increased to 12 days for 5F vaccine (table 2). The pyrexia was milder for 5F vaccine in comparison with the original vaccine. As is shown

Table 3. *Incidence of rash and clinical observation*

Type of vaccine	No. of children	Rash: Mild (%)	Rash: Severe (%)	Rash: Mean onset (days)	Rash: Mean duration (days)	Koplik spot (%)	Cough (%)	Coryza (%)	Conjunctivitis (%)	Tonsillitis (%)	Convulsion (%)
Sug. 82	85	39 (46)	19 (22)	10·5	4·2	1·2	10	54	36	8	—
5F 100	70	22 (31)	2 (3)	12·5	2·1	—	6	22	5	4	—

Table 4. *Serologic responses in initially seronegative children*

Type of vaccine	No. of sera tested	Children with maternal antibody	Seroconversion convertors/vaccinated	%	GMT*
Sug. 82	85	6	76/79	96	7·0
5F 100	70	6	61/64	95	6·1

* Geometric mean titre: HI titre $\log_2$.

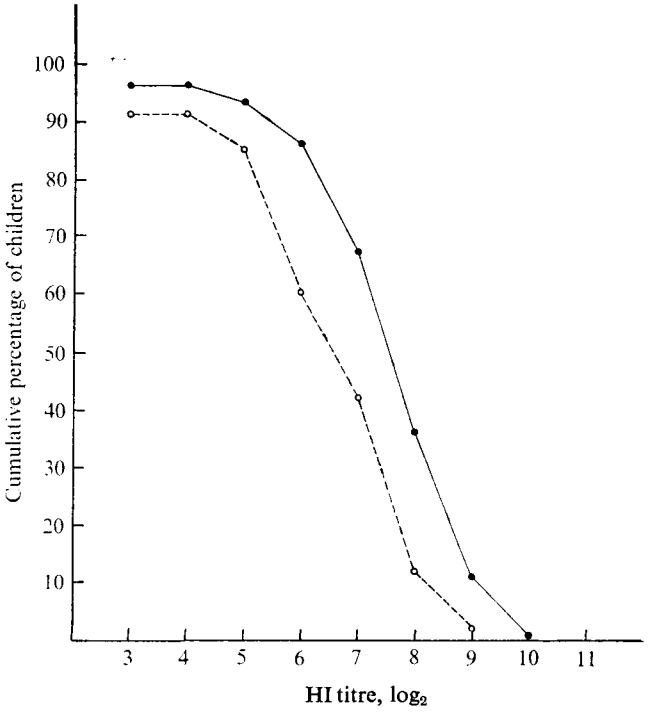

Fig. 1. HI antibody response in vaccinated children initially seronegative. ●—●, Sugiyama passage 82; ○– – –○, 5F 100 vaccine.

in Table 2, only 71 % of those given 5F vaccine reacted with fever. Of this number 64 % had a mild fever (37–38° C.), 5·7 % had shown a temperature of 38·1–39° C. Only one child had a fever of 39·5° C. Mean duration of pyrexia was 1·5 days and mean duration of maximum temperature was only 1 day.

Pyrexia was more severe in children immunized with the original vaccine. In

https://doi.org/10.1017/S0022172400023482 Published online by Cambridge University Press

this group 54% of children showed a mild rise of temperature, 19% had fever of 38·1–39° C. and 3·5% a fever of over 39° C. The mean duration of pyrexia, 4·22 days, was much longer than that of the first group; the mean duration of maximum temperature was 2·6 days.

The percentage with rash was lower in children immunized with 5F vaccine. It consisted of sparse and tiny exanthema which appeared on average 12·5 days after vaccination and faded in 2 days. The morbiliform rash of the original vaccine was usually indistinguishable from natural measles, appearing about 10 days after vaccination and lasting 4 days. Koplik's spots were not observed in those given 5F vaccine. The percentage of other symptoms such as cough, coryza, conjunctivitis and tonsillitis was also reduced when 5F vaccine was used (Table 3).

Virulence for baby hamsters

The symptoms of illness in some suckling hamsters, inoculated with Sugiyama original vaccine, were observed 14–18 days after inoculation. In three successive experiments the ratio of baby hamsters showing signs of encephalitis was 1:5. Measles virus was isolated in all cases from the harvested brain in Vero cells. The suckling hamsters inoculated with 5F vaccine did not show any sign of illness and in three experiments measles virus was not isolated from their brains.

Serological findings

The seroconversion rate in both groups was 95% (Table 4). Although the cumulative titre distribution curves for the two vaccines were parallel (Fig. 1), the mean antibody titre was about 1 $\log_2$ lower in children immunized with 5F vaccine.

DISCUSSION

The Sugiyama vaccine prepared in Japan at the 73rd passage in CK cells was one of the two further attenuated vaccines which have been considered by the Japan Measles Vaccine Research Commission (Shishido, 1969) to be safe and able to be used without gammaglobulin or prior inoculation of killed vaccine. The seed material of Sugiyama virus received through the courtesy of Dr S. Hashizume of Chiba Serum Institute, Japan was at passage 78 in primary CK cells. After three passages in our laboratory in the same cell system a seed lot was prepared. Five successive batches, each of over one million doses, were then produced at passage 82 in primary young CK cells. About 6 million doses of this vaccine have so far been used throughout Iran mainly in rural regions in children of 1–5 years without any untoward reaction (Manteghi, 1971). Fever and clinical symptoms observed after use of this vaccine were mild, as we have described previously (Mirchamsy *et al.* 1970, 1971). Seroconversion also was 95–97% with a mean titre of 7·5 $\log_2$ (Mirchamsy *et al.* 1971). The only remark made by many physicians about this vaccine was that the type of rash and its occasional intensity was sometimes similar to the natural measles.

The new seed virus 5F, also kindly supplied by Dr Hashizume, has been propagated twice in our laboratory in young CK cells in order to produce a seed lot virus. A small batch of vaccine was then produced with this new seed virus and a

comparative study was undertaken in order to assess the changes which had resulted from clonage of Sugiyama attenuated virus. From data presented in this report and those already reported by Myamura *et al.* (1971) one can assume that the 5F strain of Sugiyama virus is a further attenuated strain of measles virus. The thermal reaction in vaccinees was lower in comparison with the parent vaccine, the rash was sporadic and consisted of tiny spots which faded in 2 days. The seroconversion rate was not changed and remained at 95%. The mean antibody titre was decreased to 6·1 or 1 $\log_2$ less than the mean titre in vaccinees with the parent vaccine.

The difference of 1 $\log_2$ in mean antibody titre of the two vaccines is not significant and cannot diminish the duration of immunity. The mean antibody titre of the well-known attenuated measles vaccine strains such as Schwarz, Leningrad-16, Biken CAM, ESC, and AIK strains, in different field trials, have been found to be 6·1, 6·4, 5·1, 6·3 and 6·6 respectively (Bolotovsky & Zetilova, 1968; Okuno *et al.* 1971; Shishido, 1969; Makino *et al.* 1973). It is also a fact that the high neutralizing antibody titre, normally observed 4–6 weeks after natural measles infection or after immunization with live vaccine, will decrease gradually to a low titre which may persist for many years or even during life.

The lack of pathogenicity for suckling hamster was observed in 5F cloned virus; the parent virus was slightly virulent for baby hamster.

We thank Dr Hashizume and Professor M. Matumoto for providing seed viruses and for comments on the manuscript.

REFERENCES

BOLOTOVSKY, V. M. & ZETILOVA, L. P. (1968). Comparative study of the reaction-causing properties and the immunological and epidemiological effectiveness of Leningrad-16 and Schwarz live measles vaccines. *Bulletin of the World Health Organization* **39**, 293–8.

CHUMAKOV, M. P., VOROSILOVA, M. K., GRACEV, V. P., DZAGUROV, S. G. & SINEAK, K. M. (1967). Le vaccin vivant antirougeoleux de la souche très attenuée E.Sch.Ch. (Edmonston-Schwarz-Chumakov) preparé par l'Institut de poliomyelite et d'Encephalites de l'Union Soviétique. *Archives Roumaines de Pathologie Expérimentale et de Microbiologie* **27**, 17–33.

MAKINO, S., SASAKI, K., NAKAMURA, N., NAKAGAWA, M., KASAHARA, S., MIZUNOE, K., HASHIMOTO, T., EHIHARA, T., NAKAZAWA, S., OKA, H., SATO, H., WATANABE, O., NIINO, K., KOGA, F., ONUMA, M., KAGAMI, K., KIMURA, M., ISHIGURO, Y., YOSHIDA, Z. & FUGII, H. (1973). Field trial with a further attenuated live measles virus vaccine. *Japanese Journal of Microbiology* **17**, 75–9.

MANTEGHI, A. (1971). The epidemiological survey of measles in Iran following mass vaccination. *Ministry of Health Report* **49**, 1–49.

MATUMOTO, M., MUTAI, M., SABURI, Y., FUJII, R., MINAMITANI, M. & 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**, 433–48.

MIRCHAMSY, H., NAZARI, F., STELLMAN, C. & ESTERABADY, H. (1968). The use of dried blood absorbed on filter paper for the evaluation of diphtheria and tetanus antitoxins in mass surveys. *Bulletin of the World Health Organization*, **38**, 665–71.

MIRCHAMSY, H., SHAFYI, A., BASSALI, Y., BAHRAMI, S. & NAZARI, F. (1970). A comparative study of two live measles vaccines in Iran. *Journal of Hygiene* **68**, 101–10.

MIRCHAMSY, H., SHAFYI, A., BAHRAMI, S., NAZARI, P., MIRZADEH, M. & BASSALI, Y. (1971). Mass immunization of children in Iran with live attenuated Sugiyama measles virus adapted to calf kidney cell culture. *Japanese Journal of Experimental Medicine* **41**, 39–48.

Mirchamsy, H., Razavi, J. & Ahourai, P. (1972). Pathogenesis of vaccine strains of measles virus in suckling hamsters. *Acta Virologica* **16**, 77–9.

Myamura, K., Yoshizawa, S., Tania, T., Sakai, K., Hashizume, S., Okuni, H., Kawana, R. & Kimura, M. (1971). Further attenuated measles virus strain Sugiyama, having lower ceiling temperature, derived from Sugiyama original strain by elution from aluminum phosphate and limiting dilution of the eluate. *19th Annual Meeting of the Society of Japanese Virologists.*

Okuno, Y., Ueda, S., Kurimura, T., Suzuki, N., Yamanishi, K., Baba, K., Takahashi, M., Konobe, T., Sasada, T., Onishi, K. & Takaku, K. (1971). Studies on further attenuated. live measles vaccine. VII. Development and evaluation of CAM-70 measles virus vaccine. *Biken Journal* **14**, 253–8.

Shishido, A. (1969). A field trial of further attenuated live measles virus vaccines in Japan–1968. *Japanese Journal of Medical Science and Biology* **22**, 191–200.