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Evaluation of live attenuated measles vaccines prepared in human diploid cells for reimmunization

By H. MIRCHAMSY, A. SHAFYI, P. NAZARI, M. P. ASHTIANI and A. SASSANI

Razi State Serum Institute, P.O. Box 11365–1558, Tehran, Islamic Republic of Iran

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SUMMARY

Two live attenuated measles vaccines developed in baby calf kidney cells, a similar vaccine produced in chick embryo chorioallantoic cells and five vaccines prepared from human diploid cells (HDC) have been studied by subcutaneous injection in groups of susceptible and immune children in three field trials. The results indicated that the vaccine developed in chick embryo cells which caused mild clinical reactions, had induced a lower seroprotection rate in susceptible children and only a low rise in hemagglutination-inhibition (HI) antibody titre in previously immunized children. The serological responses induced by vaccines developed in HDC or in calf kidney cells were satisfactory in both susceptible and immune children. The superiority of HDC grown measles vaccine for revaccination is discussed.

INTRODUCTION

In two separate field trials to evaluate two live attenuated measles vaccines, it was found that susceptible children vaccinated with a further attenuated vaccine showed few clinical symptoms but low rates of HI anti-body were induced. The same vaccine provoked a poor anamnestic response in previously immunized subjects. These experiments were repeated in a comparative study with five attenuated measles vaccines produced in HDC. The clinical and serological responses of these vaccines in susceptible infants have been reported before (Mirchamsy *et al.* 1977). The boosting effects of these vaccines are discussed in this report.

METHODS AND MATERIALS

Subjects and vaccines strains

The study population for the first and second field trials were 523 and 193 children, respectively, from five villages near the Razi Institute. For the first survey the Denken lyophilized vaccine containing the Sugiyama strain at the 72nd passage in baby calf kidney cells (CK), lot no. 2A was supplied by the Chiba Serum Institute in Tokyo and the Biken lyophilized vaccine, lot no. 6803, was received from the Research Institute for Microbial Diseases, Osaka University. For the second field trial the Sugiyama strain at its 82nd passage in CK, produced at the

Razi Institute was used. Children were immunized subcutaneously with 500 $TCID_{50}$ of vaccine in a volume of 0·25 ml as previously reported (Mirchamsy *et al.* 1970, 1974). For the third field trial several villages in the Caspian Sea area in the north of the Islamic Republic of Iran were selected. Healthy children aged 10 months to 6 years, evenly distributed between the two sexes and without a known history of measles infection, were randomly allocated to one of the five groups.

Measles vaccines made in HDC

Five attenuated measles virus strains were isolated in MRC-5HDC human diploid cells directly from the original vaccines and propagated to make experimental vaccines. Details have been given elsewhere (Mirchamsy *et al.* 1977). Briefly their passage histories were:

AIK-C vaccine, lot no. TV12, supplied by Dr S. Makino of Kitasato Institute, Tokyo, was an Enders–Edmonston virus which had been further attenuated by Makino, Sasaki & Nakamura (1973*a*,*b*). The history of its attenuation in sheep kidney cells and its adaptation to chick embryo culture has been published (Mirchamsy *et al.* 1977).

Biken vaccine developed by Okuno *et al.* (1960) was derived from the Toyoshima strain of measles virus propagated in the amniotic cavity of developing chick embryos.

Leningrad-16 *vaccine*, lot no. 69-6 was supplied by the Moscow Research Institute as lyophilized virus.

Schwarz vaccine (*Rouvax*), lot no. Y0616, manufactured by the Mérieux Institute, Lyon, France was obtained through the Ministry of Health, Tehran.

The Sugiyama strain was adapted by Matumoto *et al.* (1962) to CK cells. A cold variant of this strain was isolated after elution of virus from aluminium phosphate and purification by limiting dilution (K. Myamura *et al*, pers. com.). This strain, called 5F100, was supplied by Dr S. Hashizume of the Virus Department, Chiba Serum Institute, Japan. The virus was isolated from lot 53-10 of vaccine produced after two further passages of 5F100 virus in CK cells at the Razi Institute.

Vaccine production

The above strains were subcultured (0·1–0·01 plaque-forming units/cell) five times in MRC-5 cells at 33 °C (except the Sugiyama strain which was incubated at 30 °C). The final medium for virus culture was Parker's 199 containing 0·2% gelatin, kanamycin 50 μg/ml and neomycin 50 μg/ml. Five experimental batches of vaccines were lyophilized and their infective titres were found to be between $10^{3.7}$ and $10^{4.2}$ $TCID_{50}$/dose. The safety of each batch was assessed by the monkey test using *Cercopithecus aethiops* monkeys (imported from Chad) which had been shown to be negative for measles HI antibody. Two monkeys were inoculated by both the intrathalmic and intraspinal routes with each vaccine. The animals were killed after 3 weeks and the brain and spinal cords were examined for lesions. None were found. The absence of virulence of each strain was confirmed by looking for evidence of contact infection. Two monkeys were inoculated with 5000–10000 $TCID_{50}$ of each experimental vaccine and were kept in separate cages with two

uninoculated monkeys free from measles antibody. One month later the sera of all contact monkeys were free from measles antibody.

Serological testing

Blood samples were collected from the children immediately before immunization and 30 days later. The blood was collected from finger pricks on filter paper disks as described previously (Mirchamsy *et al.* 1968). Details of HI test have already been reported (Mirchamsy *et al.* 1977). A fourfold or greater rise in HI titre was accepted as a significant increase.

RESULTS

Our first and second trials have shown that vaccines based on the Sugiyama strain either received from Japan or manufactured locally initiated seroconversion rates of 91·7 and 96·0% in susceptible children with a geometric mean titre (GMT) of 7·38 and 7·0 $\log_2$ respectively. It was also noted that a fourfold or greater increase in antibody titre was found in 50 and 79% of subjects with previous history of measles (by natural infection or by vaccination) following inoculation of these two vaccines (Table 1). There was no change in HI titre following vaccination in 35 and 21% of children while a reduction of HI titre was noticed in 15% of children who were immunized with the Sugiyama vaccine imported from Japan. With the Biken vaccine the seroconversion rate in susceptible children was 78·5% and the GMT was 5·89 $\log_2$. In immune children, while only 28% showed an increase of HI antibody following revaccination, in 58% of vaccinees no changes in antibody titre occurred within 1 month after vaccination and in 14% of subjects a decrease in HI titre was noticed. Therefore a difference in potency between the Biken and Sugiyama vaccines was evident ($P < 0{\cdot}05$). The clinical reactions following injection of the Biken vaccine were mild with a rash appearing in only 8·7% for the Biken vaccine compared with 26·5% following administration of the Denken vaccine. The seroconversion rate and the GMT following the Biken vaccine were also lower than those obtained after the use of the Denken vaccine (Mirchamsy *et al.* 1970).

In the third field trial, following vaccination with the five vaccines produced in HDC, the seroconversion rates in susceptible children were between 95 and 100% with a GMT varying between 5·2 and 6·5 $\log_2$. In those with previous history of measles, a fourfold or higher increase in HI titre in 79, 86 and 87% were noted for the Schwarz, AIK-C and Sugiyama strains respectively. In contrast, the Biken and Leningrad-16 measles viruses also adapted to HDC showed increases of HI antibody in 48 and 47%. Adaptation of 3 out of the 5 strains of attenuated measles virus to HDC has apparently increased the immunogenicity of these viruses because a fourfold increase in HI antibody was noted in most of the reimmunized children 1 month after revaccination at which time the decrease in titre was nil or negligible.

Table 1. *Serological response following immunization and reimmunization with different types of live attenuated measles vaccines*

	Susceptible children			Immune children			
					HI Titre (%)		
Type of vaccine	No. tested	Seroconversion (%)	GMT* $\log_2$	No. tested	Increase	No change	Decrease
Sugiyama CK72 (Japan)	243	91·7	7·38	20	50	35	15
Sugiyama CK82 (Iran)	85	96·0	7·0	14	79	21	0
Biken (Japan)	135	78·5	5·89	14	28	58	14
Sugiyama 5F100/ HDC-5	158	95·6	5·7	24	87	8	5
Biken/HDC-5	140	95·5	5·6	64	48	42	10
AIK/HDC-5	133	100	5·2	51	86	14	0
Schwarz/HDC-5	178	96·7	6·5	58	79	21	0
Leningrad 16/ HDC-5	163	100	6·4	51	47	53	0

* GMT, Geometric mean titre.

DISCUSSION

Since the rates of seroconversion and protection induced by the usual attenuated virus vaccines was found to be insufficient (Linnemann *et al.* 1972; Cherry *et al.* 1972, 1973), the idea of revaccinating children with attenuated vaccines was suggested by Immunization Practices Advisory Committee of the United States of America in 1977 (CDC, 1977). The committee's advice was to 'give the vaccine to all school-children from kindergarten to high school, inclusive, without reference to previous history of vaccination or natural infection' and 'administration of the vaccine to children who have already had the natural disease or a dose of live virus vaccine (for which there is no record) can do no harm and may give a boost to their immunity'. According to the health authorities the reason for the increase in measles cases and its more widespread occurrence in 1986 in the United States was partly due to vaccine failure (CDC, 1986). The widespread recurrence of measles epidemics can play the role of booster for previously vaccinated children. However, it is evident that potent vaccines are needed for controlling the measles epidemics which are not rare among previously immunized communities. A suitable vaccine should induce a high seroconversion rate and a geometric mean antibody titre high enough to persist for many years and, preferably, for life. With some exception, most live attenuated measles vaccines presenting in use are produced in chick embryo fibroblast (CEF) cells. Whether attenuated measles virus retains a stable immunogenicity after repeated passages in CEF is a very important facet of the production of vaccines. With the Edmonston strain, further attenuation was not detected at its 53rd passage in CEF (Gaffe & Laurence, 1961) and only became evident after over 90 passages (Schwarz, 1962). The Beckenham 20 strain, a derivative of the Edmonston B virus, was exceptionally in being suspected to have reverted to full virulence during some of its passages in CEF and the related vaccine was therefore withdrawn from the market leading (Article 1969).

The Chinese further attenuated strain Chang 47 retained its immunogenicity from the 25th up to the 56th passage in CEF while for another strain, Chang 12, stability was noted only between the 16th and 46th passage (Hsin-Chun, 1975). Besides the fluctuations in stability which may impair the immunogenicity of a vaccine strain, the number of virus particles injected into a susceptible host has a considerable effect on the evolution of the clinical reactions and the level of immunogenicity. Böttiger *et al.* (1973) have observed that 1000 $TCID_{50}$/dose of Schwarz vaccine (which is a recommended dose according to international standards, did not induce seroconversion in 15% of susceptible children, 5000 $TCID_{50}$/dose induced substantial amounts of HI antibody in 95% of susceptible vaccinees and causes only a moderate clinical reaction. Finally when 12000 $TCID_{50}$/dose of the same vaccine was given, the incidence of fever and rash were, respectively, about two and four times higher in previously immune children. In a comparative study of the Schwarz vaccine made in CEF and the HDC Edmonston–Zagreb vaccine used to immunize 4-to 24-month old children, Sabin *et al* (1984) found that after the HDC vaccine, more children had increased titres (67%) than decreased titres (13%) in the 4-to 6-month age group while 53% had increases versus 16% with reduced levels in the 12-to 24-month age group. After

the CEF vaccine, only 16% showed an increase versus 25% with a decrease in the 4-to 6-month age group and 34% with an increase versus 52% with a decrease in the 12-to 24-month age group. The authors concluded that the Schwarz strain of measles virus is unsuitable for immunization of infants younger than 6 months, while very high seroconversion rates were obtained with the Edmonston-Zagreb strain, developed in HDC, in 5- and 6-month-old infants (89 and 100% respectively), 14 weeks after vaccination. Our findings with the five strains of measles viruses adapted to HDC and inoculated into both susceptible and immune children demonstrates the superiority of vaccines produced in HDC. The results shown in Table 1 indicate that Schwarz, AIK-C and Sugiyama measles vaccines produced in HDC initiate 95·6–100% seroconversion rates in susceptible children and provoked 79, 86 and 87% increase in titre as against 0·0 and 5% decreases respectively. The Biken strain adapted to the amniotic cavity of the developing chick embryo only induced seroconversion in 78·5% of susceptible children and an increase of 28% HI antibody titre in immune infants. The Biken vaccine has been widely used in Japan and in Thailand (Ueda *et al*, 1966) but we understand that, in most field trials, this vaccine has been given either by inhalation or after administration of a killed measles vaccine. In both cases the reactions were mild or inapparent. In our study also the clinical reactions due to the Biken vaccination were mild. The vaccine manufactured from the same strain and adapted to HDC demonstrated a seroconversion of 95·5% and an increase in titre in 48% and a decrease in 10%. The Leningrad-16 strain which has apparently been adapted to HDC, gave 100% seroconversion, in titre in 47% increase versus a decrease in 0% of immunized subjects.

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