CLINICAL RESEARCH

Further field testing of the more heat-stable measles vaccines in Cameroon

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Abstract

Two of the more heat-stable measles vaccines were field tested in Cameroon. Both maintained the minimum required infectivity titre and the ability to induce seroconversion after storage unreconstituted at 37°C for 14 days. One of the vaccines, studied after reconstitution, maintained its ability to induce seroconversion after reconstitution and storage at 25°C for 48 hours and at 37°C for at least four hours.

The increased heat stability of the studied vaccines will not eliminate the need for a well-monitored system of vaccine conservation and distribution but will ease the rigid cold-storage requirements of conventional measles vaccines.

Introduction

Measles is an important cause of death among children in the developing world, and case fatality rates may be as high as 7%.[1] Efforts to control the disease are often hindered by administration of vaccines inadvertently inactivated by heat because of logistic problems such as unreliable or non-existent cold-storage equipment or inadequate maintenance and monitoring systems for conserving vaccine.[2]

The development of the more heat-stable measles vaccines during the past few years has helped to overcome some of the problems associated with cold storage. At 2°-8°C these improved vaccines maintain the minimum required infectivity titre (10^3 $TCID_{50}$/dose) for more than two years.[3 4] Their stability is even more pronounced at higher temperatures. Rimevax, a more heat-stable measles vaccine studied by us in 1979, showed negligible loss in virus titre during seven days of storage at 25°C and induced seroconversion in 122 (92%) of 132 children vaccinated.[5]

During the past year a second generation of Rimevax, stable at higher temperatures, and a second more heat-stable measles vaccine, Attenuvax, have become available. Both vaccines have been shown by their manufacturers to maintain the minimum required infectivity titre after 14 days of storage at 37°C.[3 4] In addition, reconstituted Attenuvax reportedly maintains this titre after 48 hours at 24°C and after seven hours at 37°C.[4] We assessed these two improved vaccines under controlled field conditions in sub-Saharan Africa by determining seroconversion rates after storage at 37°C for periods up to 14 days. We also determined seroconversion rates to reconstituted Attenuvax stored at 25°C for periods up to 48 hours and at 37°C for periods up to six hours. Finally, based on our results we formulated guidelines for transport and use of these more heat-stable vaccines in the developing world.

Methods

Study population—Children 9-24 months of age living in rural, south-central Cameroon were selected for study from children attending routine immunisation clinics. Children with a history of measles or measles vaccination were excluded. Before enrolment the study was explained to the accompanying parent, whose consent was obtained.

Seroconversion after storage at 37°C—Ten dose phials of Rimevax and Attenuvax from routine manufacturers' stocks and within three

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months of manufacture were transferred from storage at −20°C to a dark container and maintained at 37°C in a standard bacteriology incubator. Temperature was monitored with a recording thermometer. After 24 hours, and at varying time intervals to a maximum of 312 hours, the lyophilised vaccine was reconstituted with diluent at room temperature (about 25°C) and immediately administered to the study children. At the end of the 312-hour storage period at 37°C two phials of each vaccine were returned to storage at −20°C for later titration. Vaccine virus infectivity titration was performed by standard procedures at the Centres for Disease Control. Capillary blood specimens were obtained by finger puncture before and 30 days after immunisation. Blood was collected on absorbent filter-paper discs as described by Mathews,[6] dried for 24 hours, and stored sealed with a desiccant at −20°C until analysis. Determinations of measles haemagglutination inhibition antibody were performed by techniques of Hierholzer and Suggs[7] and Norrby.[8] Seroconversion was defined as a rise in titre of measles haemagglutination inhibition antibody from seronegative (<1/10) to ⩾1/20. Children with a haemagglutination inhibition antibody titre of ⩾1/10 in the prevaccination blood specimen were considered immune at the time of vaccination and not included in the analysis.

Seroconversion after storage of reconstituted vaccine at 25°C and 37°C—Ten dose phials of Attenuvax from routine manufacturers' stocks and within three months of manufacture were removed from storage at −20°C and reconstituted with diluent at room temperature (about 25°C) using sterile needle and syringe. The reconstituted vaccine was stored in a dark container at 25°C for up to 48 hours and at 37°C for up to six hours. After storage intervals of 24 and 48 hours at 25°C and of four and six hours at 37°C the reconstituted vaccine was removed from storage and immediately administered to study children. Blood specimens were obtained before and 30 days after vaccination and analysed as previously described.

Revaccination of study population—The study was performed during a period when the incidence of measles transmission was low. At the time of collection of the second capillary blood specimen all study children were revaccinated with vaccine that had been kept frozen at −20°C until reconstitution and use. Parents were questioned at the time of collection of the second blood specimen to ascertain that study children had not had measles-like disease during the interval since vaccination.

Results

Of 522 children enrolled in the study, 427 remained for analysis after exclusion of those who failed to comply and those with prevaccination measles haemagglutination antibody titres of ⩾1/10. Of 137 seronegative children vaccinated with Rimevax after storage at 37°C for up to 14 days, 134 (98%) seroconverted; 191 of 198 (96%) seronegative children vaccinated with Attenuvax stored at 37°C for up to 14 days seroconverted (table I). The titre of both Rimevax and Attenuvax was $>10^{3\cdot5}$ $TCID_{50}$/dose after the 312-hour storage period at 37°C.

TABLE I—*Seroconversion to Rimevax and Attenuvax among children aged 9-24 months, Cameroon 1979, after storage of unreconstituted vaccine at 37°C*

Duration of storage at 37°C (hours)	Rimevax		Attenuvax	
	No of seronegative children vaccinated, with 30-day follow-up	No (%) seroconverted	No of seronegative children vaccinated, with 30-day follow-up	No (%) seroconverted
24–48	24	23 (96)	48	46 (96)
49–72	15	15 (100)	28	28 (100)
73–96	19	19 (100)	23	22 (96)
97–120	16	16 (100)	10	10 (100)
121–144	14	14 (100)	24	24 (100)
145–168	16	16 (100)	13	13 (100)
169–192	16	15 (94)	23	23 (100)
289–312	17	16 (94)	29	25 (86)
Total	137	134 (98)	198	191 (96)

Among seronegative children vaccinated with reconstituted Attenuvax stored for up to 48 hours at 25°C, 19 out of 20 (95%) seroconverted. Among seronegative children vaccinated with reconstituted Attenuvax stored for four hours at 37°C, 12 out of 13 (92%) seroconverted, while among those vaccinated with reconstituted Attenuvax stored for six hours at 37°C, 31 out of 40 (78%) seroconverted (table II). Though there was a decrease in the seroconversion rate to reconstituted Attenuvax that had been stored at 37°C for six hours as compared with that stored at 37°C for four hours, this decrease was not statistically significant (p = 0·19, Fisher's exact test).

TABLE II—*Seroconversion to Attenuvax in children aged 9-24 months, Cameroon 1979-80, after storage of reconstituted vaccine at 25°C and 37°C*

Duration of storage (hours)	Storage temperature (°C)	No of seronegative children vaccinated, with 30-day follow-up	No (%) seroconverted
24	25°	19	18 (95)
48	25°	20	19 (95)
4	37°	13	12 (92)*
6	37°	40	31 (78)*

*p = 0·19 (Fisher's exact test).

Discussion

Unreconstituted Rimevax and Attenuvax maintained both the minimum required infectivity titre and the ability to induce seroconversion after storage at 37°C for 14 days. Reconstituted Attenuvax maintained its ability to induce seroconversion after storage at 25°C for 48 hours and at 37°C for at least four hours.

The increased stability of these vaccines will overcome many of the cold-storage and logistic problems in transporting and using measles vaccine in the developing world. Stability of unreconstituted measles vaccine at 2°-8°C permits storage for up to two years in the refrigerator. Stability at higher temperatures permits transport of measles vaccine at ambient temperature provided that the vaccine is continuously monitored so that the temperature and time limits of stability are not exceeded. Though transport at ambient temperature is not recommended routinely, it may inadvertently occur when the chain of cold-storage facilities fails during transport of vaccines, and it may be necessary to ship measles vaccine from regional storage depots to remote areas without refrigeration.

Stability of the more heat-stable measles vaccines after reconstitution permits use of reconstituted measles vaccine for at least four hours at a temperature of 37°C and for 48 hours at 25°C. As with all biological preparations, reconstituted heat-stable measles vaccines must be reconstituted using standard sterile technique. Because of the risk of bacterial contamination during reconstitution or aspiration and resultant bacterial proliferation, the reconstituted vaccine should be stored away from direct light at a temperature between 2°C and 8°C when not in use and should be given within eight hours of reconstitution.

The increased stability of these vaccines does not eliminate the need for a well-monitored system of vaccine conservation and distribution. Their increased stability does ease the rigid cold-storage requirements of conventional measles vaccines, thereby decreasing wastage due to temporary failure of cold storage, facilitating transport to remote areas, and prolonging the period of use after reconstitution at immunisation sites. The World Health Organisation's minimum criteria of stability for the more heat-stable measles vaccines require that at the time of manufacture the vaccine should retain a virus infectivity titre of 10^3 $TCID_{50}$/dose after seven days of storage at 37°C.[9] Stability of the two vaccines studied here was greater than this and varied with the initial virus infectivity titre of the lot. Hence to establish temperature and time limits for use of a measles vaccine designated as more heat stable, manufacturer's stability data and date of manufacture of each lot must be known.

Using the trade names Rimevax and Attenuvax is for identification only and does not imply endorsement by the Public Health Service of the US Department of Health and Human Services.

Requests for reprints should be addressed to the International

Health Programme Office, Centres for Disease Control, Atlanta, Georgia 30333 USA.

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Are HLA antigens important in the development of alcohol-induced liver disease?

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Abstract

The prevalences of 10 HLA-A and 16 HLA-B antigens were determined in 50 patients with alcoholic cirrhosis and 120 alcoholic patients without cirrhosis and compared with those in a control group of 550 healthy subjects from the same geographical area. B40 was absent in the patients with cirrhosis but was found in 18 (15%) of the patients without cirrhosis ($p = 0.0087$). No other association was noted.

It is concluded that there is no good evidence to date of an association between HLA antigen state and susceptibility to alcohol-induced cirrhosis.

Introduction

While the quantity of alcohol consumed and duration of consumption probably play a major part in the pathogenesis of alcohol-induced cirrhosis, other as yet poorly identified factors exist since most alcoholics do not develop cirrhosis despite very heavy drinking.[1] One possible factor—genetic predisposition—has been studied in some detail by investigation of the HLA polymorphism among alcoholics. The results have so far been conflicting, some workers showing no evidence of any association between a particular HLA antigen and alcohol-induced cirrhosis[2] while others have shown possible linkage with A28, B8, B13, and B40,[3-5] all in fairly small groups. In the present study we investigated a larger number of patients to assess their antigen state and determine whether there was any association with alcohol-induced cirrhosis.

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Subjects and method

Over two years we studied 170 consecutive alcoholics, all of whom had been admitted for detoxification or treatment of alcoholism. Fifty patients showed signs of chronic liver disease, and histology of liver biopsy specimens in 39 showed cirrhosis; in the remaining 11 liver biopsy was contraindicated and cirrhosis was diagnosed on clinical and biochemical grounds—for example, evidence of portal hypertension or hepatic encephalopathy. The group with cirrhosis comprised 15 women (mean age 58·1 ± SD 10·5 years) and 35 men (mean age 54·9 ± 9·4 years). The mean alcohol intake in this group was 194 ± 96 g/day for a mean duration of 15·3 ± 8·3 years. Of the remaining 120 patients, 26 had no biochemical evidence of liver injury or any clinical evidence of liver disease; liver biopsy was unjustified in these and they were included in the group without cirrhosis. In the remaining 94 there was biochemical evidence of liver injury and liver biopsy yielded either normal findings or evidence of liver injury short of cirrhosis, the commonest being steatosis. Of these patients, 51 were women (mean age 48·1 ± 12·8 years) and 69 men (mean age 48·4 ± 12·9 years.) The mean alcohol intake in this group without cirrhosis was 186·8 ± 115 g pure alcohol daily for a mean of 8·5 ± 7·4 years. Lymphocytes from venous blood samples were typed by a modified cytotoxicity technique[6] for specificity to 10 HLA-A and 16 HLA-B antigens. ABO blood groups were determined on the blood samples and compared with those of a large control series from the same geographical area.

Serum samples were tested for the presence of antinuclear, smooth-muscle, mitochondrial, parietal cell, and reticulin antibody by fluorescence antibody methods. Tests for the presence of hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) were carried out in most cases. Relative risks were calculated according to the method of Haldane.[7]

Results

Table I gives the prevalences of the HLA-A and B series antigens in alcoholic patients with and without cirrhosis and the controls together with the significances of the differences between the three groups. B40 was absent in the patients with cirrhosis but present in 18 (15%) of those without cirrhosis and in 64 (11·6%) of the normal controls. No other noticeable differences were apparent, in particular with regard to A28, B8, and B13.

Table II gives the prevalences of the ABO blood groups in patients and controls; there was no significant difference between the three groups. Four patients showed very low titres of antinuclear antibodies; two had antibodies to smooth-muscle, one to reticulin, and six to