Chorioallantoic Membrane Measles: Development and Clinical Evaluation of a New Further Attenuated Measles Vaccine

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A new, further attenuated measles vaccine, developed by propagation of Edmonston B measles virus in the chorioallantoic membrane in ovo, has been clinically evaluated in children and found to compare favorably with a commercially available product. The vaccine is prepared in chick embryo tissue culture and appears to possess noteworthy stability at 4 C.

The adaptation of the Edmonston B strain of measles virus (3) to growth on the chorioallantoic membrane (CAM) of the embryonated hen egg has been reported previously from these laboratories (1). The 68th and 81st passage levels of CAM measles virus were used as seed to produce vaccines in 1966, and were tested in small clinical trials (unpublished data) which indicated that, if an acceptable level for antigenicity and more diminished reaction rates existed, it would lie within these passage limits. This conclusion was based on the observation that the 68th passage vaccine retained characteristics of the parent Edmonston strain, whereas the 81st passage exhibited properties of over-attenuation. This paper reports on the development of vaccines at the intermediate passage levels and on their evaluation in clinical studies of 3,000 children to determine the optimal passage level for vaccine production.

MATERIALS AND METHODS

Media. Seed preparations of infected chorioallantoic membranes were harvested as 20% suspensions in 2% lactose in distilled water with 100 µg each of streptomycin and neomycin (SN) per ml. Tissue culture growth medium was Eagle basal medium in Hanks balanced salt solution with 10% calf serum and 100 µg each of SN per ml. Maintenance medium was Eagle basal medium in Earle balanced salt solution with 5% lactose and 100 µg each of SN per ml.

Preparation of vaccine levels. CAM measles seed virus was prepared in eggs as described previously (1) at passage levels 70, 72, 74, 76, and 78. For vaccine production, RIF-free chick embryo monolayers were established in glass bottles. When confluent, growth medium was removed and the monolayers were washed with phosphate-buffered saline. Seed virus at the desired passage level was diluted in maintenance medium and inoculated onto the monolayers. After an appropriate adsorption period, maintenance medium was added and bottles were incubated at 34 C. After 7 days of incubation, the bottles were subjected to a freeze-thaw cycle, and the fluids were harvested, pooled, and clarified through a membrane filter (5-µm pore size; Millipore Corp.). Filtrates were diluted, stabilized, dispensed in 0.6-ml doses, and lyophilized. Safety testing was carried out by the Quality Control Section at Lederle Laboratories as prescribed under Measles Virus Vaccine, Live, Attenuated in Regulations of the Public Health Service, Title 42, part 73, revised 1969.

Titration. All infectivity titrations were carried out in BSC-1 cells and were assayed by observation of cytopathic effect. End points were calculated as the dose infecting 50% of the tissue cultures, using serial half-log10 dilutions in eight replicate tubes. Infectivity titers ranged from 10^{4.4} to 10^{4.4} 50% tissue culture infective doses per dose after reconstitution of the lyophilized vaccines.

Serology. Serum samples were shipped under dry ice to Lederle Laboratories, allowed to thaw while being uncoded, and stored at 4 C until tested. Paired sera were treated with kaolin and cercopithecus monkey erythrocytes by a modification of Rosen's technique (7), and were tested for hemagglutination inhibition in microtiter plates against 4 units of measles antigen. Measles antigen was prepared in cercopithecus monkey kidney tissue culture by using the 25th chick embryo tissue culture passage of Edmonston B measles virus as seed. The virus pool was harvested by shaking with marble chips and homogenized by using a De Laval hydropulse homogenizer. Following homogenization, the hemagglutination titer increased approximately fourfold. Only children with a prevaccination hemagglutination inhibition antibody titer of less than 1:4 were considered susceptible. These children were required to show postvaccination titers of 1:8 or greater to be counted as seroconversions.

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Study population. Three thousand children between the ages of 9 months and 6 years in the San Jose, Costa Rica area participated in this study. Five hundred children were inoculated subcutaneously with 0.5 ml of vaccine at each of the five passage levels in a coded, randomized, double-blind study. A control group of 500 children was also inoculated with commercially obtained Schwarz strain measles vaccine in an open study. Children were observed once daily (except Sundays) for 20 days after vaccination for occurrence of fever, rash, cough, coryza, and conjunctivitis. Observations were made by personnel from the staff of the Louisiana State University International Center for Medical Research and Training, San Jose. Each child was bled on the day of vaccination and again at 4 weeks. A number of children were selected for bleeding at 6 months and 1 year postvaccination to determine the duration of hemagglutination inhibition antibody titers.

RESULTS

Study population. Table 1 illustrates the composition of the study population. An approximately even distribution of susceptible and nonsusceptible individuals, as well as males and females, was obtained in each vaccine study group. The mean age among susceptible children was 2.0 years and among the nonsusceptible was 3.5 years.

Clinical. No major complications or evidence of central nervous system involvement were noted during the 20-day observation period. The occurrence of symptoms other than rash and fever are listed in Table 2. The most significant difference (P < 0.001) in reactivity between susceptible and nonsusceptible vaccinees was coryza in the susceptible group inoculated with level 70 and conjunctivitis in susceptible groups receiving levels 70, 72, and 74.

Observations on fever and rash throughout the study period are presented in Tables 3 and 4. The total incidence of fever and rash for nonsusceptible individuals inoculated with levels 70 through 78 was subtracted to correct the incidence in susceptible children for these groups, since all were part of a double-blind, randomized study. The study conducted with Schwarz vaccine was neither blind nor randomized, and therefore the incidence in nonsusceptible Schwarz recipients only was used to obtain the corrected incidence in susceptible children for this group.

| Vaccine | Susceptible | Nonsusceptible |
|---------|-------------|----------------|
| Male    | Female      | Mean age (years) | Total no. | Male | Female | Mean age (years) | Total no. |
| 70      | 128 (51)*   | 122 (49)        | 2.12      | 250  | 131 (53)| 3.68          | 249      |
| 72      | 137 (53)    | 123 (47)        | 1.95      | 260  | 123 (52)| 3.39          | 238      |
| 74      | 131 (49)    | 139 (51)        | 2.14      | 270  | 116 (51)| 3.43          | 228      |
| 76      | 138 (51)    | 130 (49)        | 2.12      | 288  | 121 (53)| 3.61          | 229      |
| 78      | 141 (54)    | 119 (46)        | 1.90      | 260  | 134 (57)| 3.72          | 235      |
| Schwarz | 136 (54)    | 117 (46)        | 2.06      | 253  | 134 (56)| 3.53          | 241      |
| Total   | 811 (52)    | 750 (48)        | 2.05      | 1561 | 759 (53)| 3.56          | 1420     |

* Numbers in parentheses indicate percentages.

| Vaccine | Cough  | Coryza | Conjunctivitis |
|---------|--------|--------|----------------|
|         | Susceptible | Nonsusceptible | P  |
|         | Susceptible | Nonsusceptible | P  |
|         | Susceptible | Nonsusceptible | P  |

| Vaccine | Cough  | Coryza  | Conjunctivitis | Nonsusceptible | P  |
|---------|--------|---------|----------------|----------------|----|
| 70      | 50.0   | 36.5    | <0.01          | 45.8           | <0.001 |
| 72      | 46.6   | 42.4    | >0.05          | 51.2           | <0.05  |
| 74      | 49.2   | 40.3    | <0.01          | 47.8           | <0.01  |
| 76      | 46.6   | 42.3    | >0.05          | 45.3           | <0.05  |
| 78      | 48.1   | 41.3    | <0.05          | 47.2           | <0.01  |
| Total levels of 70–78 | 40.5 |        |                |                | 8.0 |
| Schwarz | 50.1   | 55.2    | >0.05          | 56.9           | >0.05  |

* Probability level by chi-square test. Incidence in each CAM-susceptible vaccine group was compared with total incidence in CAM-nonsusceptible recipients. Incidence in Schwarz-susceptible vaccinees was compared with that in Schwarz-nonsusceptible vaccinees.
TABLE 3. Percent incidence of elevated rectal temperature and corrected incidences in susceptible subjects by vaccination group

| Vaccine | Rectal temp (F) | 101 to 102.9 | 103 or greater | Total > 101 F corrected |
|---------|-----------------|--------------|----------------|------------------------|
|         | Susceptible     | Nonusceptible | Corrected      | Susceptible | Nonusceptible | Corrected |          |
| 70      | 34.1            | 15.8         | 18.0           | 10.3        | 3.6          | 7.1       | 25.1 |
| 72      | 30.8            | 14.2         | 14.7           | 10.3        | 3.0          | 7.1       | 21.8 |
| 74      | 31.4            | 16.5         | 15.3           | 15.7        | 1.3          | 12.5      | 27.8 |
| 76      | 20.7            | 18.7         | 4.6            | 6.1         | 4.0          | 2.9       | 7.5  |
| 78      | 16.2            | 15.3         | 0.1            | 3.6         | 3.9          | 0.4       | 0.5  |
| Total levels of 70-78 | | | | 2.9 | 2.5 | 0.4 | 3.7 |
| Schwarz | 22.8            | 19.5         | 3.3            |             |              |           |      |

TABLE 4. Percent incidence of rash and corrected incidences in susceptible subjects by vaccination group

| Vaccine | Susceptible | Nonusceptible | Corrected |
|---------|-------------|---------------|-----------|
| 70      | 22.5        | 3.7           | 17.6      |
| 72      | 25.3        | 6.9           | 20.4      |
| 74      | 32.3        | 1.8           | 27.4      |
| 76      | 12.4        | 5.4           | 7.5       |
| 78      | 8.8         | 6.5           | 3.9       |
| Total levels of 70-78 | | | |
| Schwarz | 8.4         | 3.0           | 5.4       |

It is evident that a reduction in the incidence of rash and fever occurred at level 76. Both levels 76 and 78 appeared comparable to the Schwarz control; however, levels 70, 72, and 74 manifested significantly higher incidences.

Fever associated with level 76 vaccine had an average day of onset at 8 days and, with Schwarz vaccine, at 9 days. The average duration of fever was 1.6 and 1.3 days, respectively. Average onset of rash was day 10 for level 76 and day 9 for Schwarz vaccine. Average duration of rash was 4.3 and 4.9 days, respectively. The other CAM vaccine levels did not differ significantly from level 76 in onset or duration of rash and fever.

Antibody titer. The apparent change in virulence at level 76 also correlates with the geometric mean antibody titers (GMT) shown in Table 5. Although all vaccines converted greater than 96% of susceptible children, the GMTs varied. Levels 70, 72, and 74 yielded the highest values, level 76 decreased to a value comparable to the Schwarz control, and level 78 resulted in the lowest titer.

Level 76, which showed further attenuation than the Edmonston parent vaccine and induced a higher GMT than level 78, appeared to be the most promising candidate for vaccine production. Serum samples obtained from individuals inoculated with level 76 and Schwarz vaccine at 6 months and 1 year postvaccination (Table 6) indicated that the decrease in antibody titer with time is consistent with previous reports in the literature (6).

Stability. During the course of this study, it was noticed that lyophilized, stabilized vaccines which had been prepared at the 68th and 81st levels showed no detectable loss of infectivity after 5 to 6 years of storage at 4 C (Table 7). Previous reports in the literature indicated that some loss at 4 C after 1 to 2 years could be expected with lyophilized attenuated Edmonston strain virus (4). When level 76 was titrated after 2 years at 4 C, there was no detectable loss in infectivity.

Expanded stability studies on freshly dried production lots of vaccine level 76 are in progress to determine the loss of infectivity at higher temperatures.

**DISCUSSION**

The data presented indicate that passage of Edmonston B measles vaccine in the chorioallantoic membrane of hen eggs has resulted, after 76 passages, in a vaccine which produces...
Table 6. Duration of antibody titer

| Vaccine | No. of sera assayed | GMT of sera obtained at: |
|---------|---------------------|--------------------------|
|         |                    | 1 Month | 6 Months | 12 Months |
| 76      | 58                  | 137     | 42       |           |
| Schwarz | 55                  | 104     | 32       |           |
| 76      | 32                  | 104     | 52       |           |
| Schwarz | 35                  | 158     | 104      |           |

Table 7. Stability of lyophilized CAM measles research vaccines at 4°C

| Year | Titer/ml at level |
|------|-------------------|
|      | 68                |
| 0    | 2.8*              |
| 2    | 3.5*              |
| 5.5  | 3.6               |
| 6    | 2.9               |

*Average of four separate titrations.

less fever and rash than the parent vaccine without significantly reducing the seroconversion rate. These characteristics compared favorably with those of a control vaccine and with those reported in the literature for further attenuated vaccines (2). Lyophilized CAM measles vaccine was stable for more than 6 years at 4°C. This property, if found to be associated with extended stabilities at higher temperatures, could prove important in light of the need for thermostable preparations of measles vaccines in developing countries (5). Efforts to determine this are currently in progress.

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