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Comparison of Three Rocky Mountain Spotted Fever Vaccines

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Growth of Rocky Mountain spotted fever (RMSF) rickettsiae in duck embryo cell (DEC) cultures and chicken embryo cell (CEC) cultures was evaluated. Experimental lots of duck embryo cell- and chicken embryo cell-grown Rocky Mountain spotted fever vaccines and a commercial lot of yolk sac-grown vaccine were compared for protective efficacy in rhesus monkeys. Incidence and magnitude of antibody response, febrile response, and rickettsemia, as well as incidence of fatalities, suggested that both cell culture-derived vaccines were more immunogenic than the yolk sac-grown vaccine.

In recent years there has been an increased incidence of Rocky Mountain spotted fever (RMSF) in the United States (1). There were 774 cased reported in 1974, a large percentage of them from the eastern United States. The mortality rate was low, due to increasing awareness by medical personnel and early administration of specific antibiotic therapy. However, prophylactic measures for protection of personnel who are at risk, because of occupational or laboratory exposure, are far from satisfactory. The presently available commercial vaccine, a formalin-treated, ether-extracted product derived from growth of rickettsiae in yolk sacs of embryonated chicken eggs (2), is relatively ineffective for protection of humans (3). Studies with RMSF vaccines prepared from formalin-killed rickettsiae grown in duck embryo cell (DEC) cultures have indicated that the DEC product was more immunogenic in guinea pigs than the commercial yolk-sac vaccine (6). Limited availability of pathogen-free duck eggs, required for development of this vaccine for human use, directed our attention toward preparation of RMSF vaccine with chicken embryo cell (CEC) cultures (7). A single lot of CEC vaccine was produced in accordance with standards for vaccines for human use (5). In the present communication the efficacy of this lot of CEC-grown vaccine for protection of guinea pigs and subhuman primates is compared with that of a lot of DEC-grown vaccine and a commercial yolk-sac product.

MATERIALS AND METHODS

Cell cultures. Preparation of cell cultures and techniques for growth and harvest of *Rickettsia rickettsii*, Sheila Smith strain, have been described (6).

Vaccines. Preparation and testing of the CEC-grown, formalin-inactivated RMSF vaccine (CEC-1974) have been reported previously (7). The DEC vaccine (DEC-1972) was processed in the same way as duck cells grown in static culture. All procedures for production and processing of the rickettsiae conformed to regulations of the U. S. Public Health Service for production and testing vaccines for potential administration to humans (5). The commercial yolk-sac source vaccine was purchased from Lederle Laboratories (Pearl River, N.Y.). Total rickettsiae were counted by the method of Silberman and Fiset (9). The method of Lowry et al. was used to measure total protein (8).

Animals. Rhesus monkeys (*Macaca mulatta*) were purchased from Prime Labs, Inc. (Farmingdale, N.J.). Monkeys (random sex and weight) were housed in individual cages and fed a commercial ration (Wayne Monkey Chow, Wayne Feed Supply Co., Gaithersburg, Md.). Rectal temperatures were recorded and blood was drawn in the morning before feeding. For 2 weeks before challenge, baseline rectal temperatures and hematologic parameters were established. Monkeys were bled for serological assays 48 h prior to vaccination, 48 h prior to challenge, and at various intervals postchallenge. Inexperienced monkeys that had no history of exposure to *R. rickettsii* and no detectable antibody constituted a nonimmune control group, and monkeys that had recovered from an experimental *R. rickettsii* infection 2 months earlier, an immune control group. Male guinea pigs (Hartley strain), weighing 300 to 350 g, were purchased from Buckberg Labs (Tomkins Cove, N.Y.).

Rickettsial determinations. Rickettsial plaque-forming units were determined by the method of Weinberg et al. (10). Heparinized blood was stored at −70 C until used for evaluation of rickettsemia by the method of Wike and Burgdorfer (11).

Serology. Microagglutination (MA) assays for rickettsial antibody were performed as described by Fiset et al. (4). The test antigen was prepared from rickettsiae grown in DEC cultures that were processed by differential centrifugation and ether extraction to remove cellular debris. Monkeys were bled 28 days after vaccination for antibody determination.

Serum samples were stored at −70 C. An MA titer of 1:4 or greater was considered to be significant.

Hematology. Blood from the femoral vein was collected in Vacutainer tubes, ethylenediaminetetraacetate (Becton-Dickinson, Rutherford, N.J.). Hematocrit values were determined with microhematocrit tubes (Yankee, Fisher Scientific, Silver Spring, Md.). Unipet diluters (Becton-Dickinson) were employed for total leukocyte counts and stained blood smears (Wright stain) for differential counts.

Guinea pig protection study. Both the CEC and DEC vaccines were titrated at dilutions of 1:10, 1:100, 1:500, 1:1000, and 1:2000 in Hanks balanced salt solution to determine the number of rickettsiae required to protect guinea pigs against lethal challenge with a fully virulent strain of *R. rickettsii*. Groups of six guinea pigs each were injected intraperitoneally with 0.5 ml of vaccine dilution and were challenged intraperitoneally 14 days later with approximately 10^6 plaque-forming units of yolk sac-grown *R. rickettsii*, Sheila Smith strain. Deaths were recorded for 2 weeks. The dilution of vaccine which conferred 50% protection was determined by probit analysis and the number of rickettsiae in that dilution was calculated.

Experimental design. Groups of rhesus monkeys were vaccinated intramuscularly with a single injection of 1 ml of serial 10-fold dilutions of the commercial vaccine or with 0.5 ml of serial 10-fold dilutions of CEC-1974 or DEC-1972. One month after vaccination all monkeys were inoculated intravenously with 10^3 plaque-forming units of chicken yolk sac-grown rickettsiae (Sheila Smith strain). The two control groups were challenged in a similar manner. Rectal temperatures were recorded in the morning and evening at 12-h intervals for 10 days. Blood from the femoral vein was collected daily for evaluation of rickettsemia and every other day for hematology.

RESULTS

Experiments were performed to compare growth of *R. rickettsii* in static or rolling cultures of CEC and DEC. Since there was a negligible increase in cell count during the 5 days of rickettsial infection prior to harvest, the total viable cell count for a representative culture prior to infection was employed for calculation of rickettsial yield per cell. Evaluation by direct count (viable and inactivated rickettsiae) and by plaque assay (viable rickettsiae only) indicated that rickettsial yield/cell was essentially the same for DEC or CEC cultures but, at least in this trial run, was somewhat reduced for static conditions of growth (Table 1).

The number of rickettsiae in the experimental lots of vaccines CEC-1974 and DEC-1972 necessary to protect 50% of the guinea pigs from death after a lethal challenge of *R. rickettsii* was determined. Results indicated the number of rickettsiae required for protection was identical (Table 2).

Mean MA antibody titers prior to challenge of groups of vaccinated or control monkeys are summarized in Table 3. None of the monkeys had an MA titer before vaccination. No reaction was detected between test MA antigen and serum obtained from guinea pigs hyperimmunized with uninfected DEC. Of 18 monkeys in-

TABLE 1. *Comparative yield of Rickettsia rickettsii cultured in CEC and DEC*

Culture	Calculated PFU[a]/cell	Calculated direct count/cell
Duck roller	6	330
Chicken roller	6	450
Duck static	2	158
Chicken static	3	260

[a] PFU, Plaque-forming units.

TABLE 2. *Characteristics of RMSF vaccines produced from growth in DEC culture, CEC culture, or yolk sac of embryonated chicken eggs*

Vaccine	Rickettsiae/ml[a]	Protein content (mg%)	Rickettsiae/$GPPD_{50}$[b]
Commercial yolk sac	NO	472	
DEC-1972	3.2×10^8	120	1.4×10^5
CEC-1974	1.3×10^8	68	1.6×10^5

[a] Direct count. NO, None observed.

[b] Number of rickettsiae conferring protection against death of 50% guinea pigs challenged with 10^6 plaque-forming units of Sheila Smith strain *R. rickettsii*. $GPPD_{50}$, Mean guinea pig protection dose.

TABLE 3. *MA antibody prior to challenge of 30-day vaccinees, RMSF survivors, or nonimmune control monkeys*

Group	Vaccine dose	Prechallenge MA titer[a]	
		Incidence	Geometric mean
DEC-1972 vaccinees[b]	10^0	6/6	1:35
	10^{-1}	4/5	1:35
	10^{-2}	2/6	1:4
CEC-1974 vaccinees	10^0	6/6	1:37
	10^{-1}	2/6	1:14
	10^{-2}	1/6	1:7
Commercial yolk sac vaccinees	10^0	1/6	1:2
	10^{-1}	0/6	0
	10^{-2}	0/6	0
RMSF survivors	None	4/4	1:320
Nonimmune controls	None	0/4	0

[a] Titer ≥1:4; no antibody titer prior to inoculation with 1 dose of vaccine.

[b] DEC vaccinees were administered the DEC-1972 reference vaccine.

jected with diluted or undiluted commercial vaccine, only one developed a significant MA titer, whereas 21 of 35 monkeys injected with DEC or CEC vaccines developed a titer of 1:4 or greater. All monkeys injected with undiluted DEC or CEC vaccine exhibited a significant antibody response.

The antigen preparation employed for vaccination and the dose of vaccine administered affected resistance to subsequent challenge (Table 4). The febrile response was lowest in groups that received undiluted vaccine; among these animals, one monkey in the DEC group, four in the CEC group, and six in the commercial group became febrile. Early onset and prolonged duration of fever appeared to be more evident in monkeys vaccinated with the commercial and CEC vaccines. Duration of fever was shorter for DEC and CEC vaccinees in undiluted groups only. For example, mean fever day values for febrile monkeys in undiluted vaccine groups were 9.4, 4.5, and 5 for the six yolk sac, four CEC, and one DEC responders, respectively. Mean duration of fever is artificially shortened and therefore less meaningful in groups in which death occurred.

Rickettsiae were recovered from blood samples of all monkeys that eventually succumbed to challenge and from 16 to 31 monkeys that survived (Table 4). Rickettsiae were first detectable at 72 to 96 h postchallenge. Magnitude of rickettsemia was related to dose and type of vaccine. Suppression of rickettsemia was evoked most effectively by immunization with DEC vaccine and least with commercial vaccine. For the DEC group, incidence of rickettsemia increased with decreased immunizing dosage. This was not observed with the groups administered the CEC or commercial vaccines. The mean duration of rickettsemia per responder did not appear to decrease with increased immunizing dosage. However, mean duration of rickettsemia is artificially shortened in groups in which deaths occurred.

All nonimmune controls died within 10 days after inoculation of challenge organisms. None of the test animals immunized with undiluted or a 1:10 dilution of CEC or DEC vaccines died. At 1:100 dilution, none of the monkeys vaccinated with DEC vaccine and only one vaccinated with CEC vaccine died after challenge. However, with the commercial vaccine, one monkey in both the undiluted and 1:10 groups and two monkeys in the 1:100 group died after challenge.

Hematologic data did not reflect significant differences between groups. There were negligible changes in hematocrit determinations; total leukocyte counts reflected normal to slightly higher than normal levels during infection. With the exception of monkeys administered undiluted DEC-1972, all monkeys including the five immune controls developed neutrophilia within 2 to 3 days after challenge. Lymphopenia was evident 3 to 9 days after challenge in all but the immune control.

DISCUSSION

In view of the increasing emphasis on purity of biologicals for human immunization, the

TABLE 4. *Response of vaccinees, RMSF survivors, and control monkeys after challenge with 10^3 plaque-forming units (PFU) of Sheila Smith strain R. rickettsii*

Monkey group	Immunization: Vaccine dose[a]	Immunization: No. of monkeys	Febrile response: No. febrile	Febrile response: Mean days: Onset	Febrile response: Mean days: Duration	Rickettsemia: No. of responders	Rickettsemia: Mean day of duration/responder	Rickettsemia: Mean peak titer (PFU/ml)	Death: No.	Death: Day
DEC-1972 vaccinees	0	6	1	8.5	5	0	0	0	0	
	−1	5	2	4.0	6.5	1	3.0	50	0	
	−2	6	5	5.8	5.4	3	2.3	30	0	
CEC-1974 vaccinees	0	6	4	3.9	4.5	1	1.0	30	0	
	−1	6	5	3.4	8.8	4	5.0	360	0	
	−2	6	5	3.5	5.8	3	3.7	1,900	1	6
Commercial yolk sac vaccinees	0	6	6	3.2	9.4	3	5.3	310	1	11
	−1	6	4	3.2	6.8	3	5.3	510	1	10
	−2	6	6	4.1	4.7	3	5.0	3,600	2	6, 8
RMSF survivors	None	4	0			0			0	
Nonimmune control	None	4	4	3.5	6.2	4	6.2	6,000	4	7, 7, 8, 10

[a] Log_{10} dilution; 0.5 ml of DEC and CEC preparations, 1.0 ml of yolk sac vaccine.

large amount of extraneous egg yolk sac protein in commercial RMSF vaccine is highly undesirable. It was postulated that a more acceptable product could be prepared from *R. rickettsii* grown in tissue culture cells. Preliminary findings indicated that a RMSF vaccine highly immunogenic for guinea pigs could be derived from growth in DEC static cultures (6); this preparation, DEC-1972, has been employed as a reference vaccine for subsequent studies. For development of preparations for human use, however, the unavailability of pathogen-free duck eggs and production requirements for high rickettsial yields directed emphasis toward utilization of CEC roller cultures. Although in the present study rickettsial yields from CEC roller cultures were somewhat superior to yields from CEC static or either type of DEC cultures, it has been our experience that rickettsial growth in either type of primary cell culture is not always consistent, possibly because of seasonal variation in the flocks used for egg production. The high rickettsial count in the reference vaccine is representative of an optimal yield for the cultural conditions.

An objective assay for evaluation of antigen content is essential for a meaningful comparison of vaccine potencies. Unfortunately, no information is available regarding RMSF antigenic content of the commercial product; examination by light microscopy failed to reveal the presence of intact rickettsiae and procedures for identifying soluble RMSF antigens have not been developed. Lack of this information renders comparison with new candidate vaccines difficult. Hence, undiluted CEC and DEC vaccines were adjusted to rickettsial concentrations considered to be physically and economically feasible, but a 1:10 dilution of these vaccines is not directly comparable to a 1:10 dilution of the commercial product.

In the present study protection against death was considered to be the most critical immunogenic parameter for determining the relative efficacy of the RMSF vaccines. By this criterion the data suggest that the tissue culture cell-source vaccines conferred better protection against RMSF than did the commercial vaccine. Antibody measurements support the conclusion that the vaccines derived from cell cultures have superior immunogenic properties. Monkeys vaccinated with undiluted CEC or DEC vaccine developed lower rickettsemia levels than those immunized with commercial vaccine. When diluted 1:10 or 1:100, little significant difference in rickettsemia levels between CEC and commercial vaccines could be observed. Only undiluted DEC vaccine evoked an immune response that effectively suppressed signs of infection. Replication of rickettsiae after challenge in vaccinated monkeys that were protected from death in the other groups suggests that vaccination induced a primary response capable of limiting development of the infectious process, and that the modified infection stimulated development of 2 fully effective secondary response. Incidence of febrile episodes was decreased for groups that received either undiluted cell-source vaccine; the most beneficial prophylaxis was associated with DEC-1972 vaccination.

Although the MA test appears to be the most reliable and consistent method for the determination of antibody to RMSF (Kenyon, unpublished observations), absence of MA titer does not indicate lack of protection. For example, with a 1:10 dilution of CEC vaccine only two of six monkeys exhibited a demonstrable MA titer, but all were protected from lethal infection. It is noteworthy however, that in no instance did a vaccinee with a MA titer succumb to RMSF after challenge, suggesting that a more sensitive immunological assay for protection against RMSF is required.

Ideal protection was not attained by a single injection with any of the vaccines, since all groups of vaccinees were less resistant than were survivors of a prior RMSF infection. Dose response of DEC vaccinees, however, suggests that optimal resistance can be achieved by increasing the antigen load. Administration of two or three injections, a procedure commonly used for immunization with killed vaccines, should be highly efficacious.

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