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Mouse Potency Assay for Western Equine Encephalomyelitis Vaccines

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A potency assay for Western equine encephalomyelitis vaccine was developed which utilized mice as the test animal instead of guinea pigs or hamsters. By immunizing several groups of mice with dilutions of the vaccine and challenging them intracerebrally with virulent virus, it was possible to determine mathematically a dose of vaccine capable of protecting 50% of the animals (ED_{50}). When log dilutions of virulent virus were used to challenge mice which were immunized with dilutions of the vaccine, there was no difference among the ED_{50} values for the dilutions of challenge virus. In a direct comparison of ED_{50} values determined from the immunization of mice and those determined from the immunization of guinea pigs, there were no differences in the rankings of the vaccines.

Randall et al. (1) described a potency test in guinea pigs for equine encephalomyelitis vaccines using a series of two 0.1-ml intradermal doses of vaccine. A vaccine was considered satisfactory if 80 to 100% of the animals were protected against an intracerebral (ic) challenge of 100 to 1,000 median lethal doses (LD_{50}).

For the potency testing of animal vaccines, the U.S. Department of Agriculture (7) has modified the vaccinations to two 0.5-ml subcutaneous (SQ) doses; for acceptable potency, 80% of the vaccinated guinea pigs must survive a 0.1-ml ic challenge which will kill at least 80% of the nonvaccinated controls.

In our laboratory the test has been further modified by vaccinating guinea pigs SQ with two 0.5-ml doses of serial fivefold dilutions of the vaccine, (i.e., 1:5, 1:25, and 1:125) and then challenging them with a constant level of virulent virus (6). Cole and McKinney (2) utilized hamsters as the test animal in an assay which was also based on immunizing with dilutions of vaccine. The results in both of these cases are reported as milliliters of undiluted vaccine effective in protecting 50% of the challenged animals (ED_{50}) and are used to compare the potencies of different lots of vaccine.

The first two tests (1, 2) are unsatisfactory for research purposes since the results give no basis for the potency comparison of different lots or types of vaccine. Although the test currently used in our laboratory and that developed by Cole and McKinney (2) can be used to compare the potencies of different vaccines, they require the use of large numbers of guinea pigs or hamsters which must be housed until the completion of the potency assay.

The development of an assay that: (i) could be completed in a shorter period of time and (ii) utilizes a lower-cost animal requiring less space would be advantageous to any vaccine development or production laboratory. Since mice rapidly develop immunity after vaccination with arbovirus vaccines, and since they are readily available, their suitability for potency testing of Western equine encephalomyelitis (WEE) vaccines was investigated.

MATERIALS AND METHODS

Vaccines and challenge virus. The method of cell culture vaccine production and the source of the challenge virus have been previously described (6).

Potency tests. The assay methods utilizing guinea pigs have been previously described (6).

For the potency assays in mice, CD-1 strain, 3-week-old white mice (Charles River Mouse Farms, Wilmington, Mass.) were divided into groups of 16, and each group was vaccinated intraperitoneally (ip) with serial fivefold dilutions of the vaccine (1:1, 1:5, 1:25, and 1:125) on days 0 and 3. This conforms to the Public Health Service requirements for Japanese encephalitis vaccine (3). A fresh vial of freeze-dried vaccine from the same lot was reconstituted and diluted for the second dose. The mice were challenged

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ic with 0.03 ml of virus suspension containing 100 to 1,000 LD_{50} of virulent virus, except in the experiment where challenge dose was compared to ED_{50}. Challenges were conducted either 7, 10, 14, or 21 days after the initial vaccination.

Nonvaccinated mice from the same group of animals were used to titrate the challenge virus. Mice were discarded at 14 days postchallenge, and titers were determined by the method of Reed and Muench (5). Vaccinated mice were held for 14 days subsequent to challenge, and the potencies of the vaccines and their confidence limits were determined by the method of Worcester and Wilson (8). In some experiments, four groups of mice were immunized with dilutions of vaccine. The potency determination requires only three consecutive dilutions, and whether the reported results were from mice vaccinated with the 1:1, 1:5, and 1:25 dilutions or the 1:5, 1:25, and 1:125 dilutions depended on the standard deviations. In every case, the results giving the smallest standard deviation are reported.

RESULTS

Spacing of the challenge dose. If the mice were challenged 7 days after the initial dose of vaccine, the gradation of the responses in relation to vaccine dose was erratic, and the ED_{50} values were high (Table 1). The ED_{50} values with challenges on day 21 were not significantly lower than the challenges on days 10 and 14, and the gradation of the response was again erratic. With challenges on days 7 and 21, groups of mice vaccinated with smaller volumes of vaccine occasionally had a greater proportion of survivors than those vaccinated with larger volumes. The challenges on days 10 and 14 gave uniform graded responses. Therefore, it was arbitrarily decided to challenge on day 14 (rather than day 10) in all subsequent experiments.

Route and level of challenge. The B-11 strain of WEE virus used as challenge virus in this study would not uniformly kill weanling mice by the ip route. For this reason, the ic route was used for all challenges.

If the level of the challenge dose affects the ED_{50}, a suitable level of challenge virus must be found if results are to be comparable. To determine if the challenge level affects the ED_{50}, mice were vaccinated with four dilutions of vaccine (96 mice per dilution) on days 0 and 3. Each group of 96 mice was separated into 6 groups of 16 mice each on day 14 and challenged with $\log_{10}$ dilutions of the virus. The results (Table 2) indicated that there were no significant differences in ED_{50} values obtained when the challenge dose contained from 32 LD_{50} to 3.2×10^6 LD_{50} of virulent virus. The

TABLE 1. *Effect of varying the day of challenge on the ED_{50} of the vaccine*

Vaccine lot	Dose of vaccine[a] (ml in 0.2 ml)				Challenge day	ED_{50} ± 2 SD (ml)
	0.2	0.04	0.008	0.0016		
A	5/16[b]	3/16	1/16	1/16	7	>0.4 —[c]
A	15/16	5/16	5/16	1/16	10	0.070 (0.025–0.202)
A	9/16	8/16	4/16	1/16	14	0.075 (0.021–0.267)
A	8/16	9/16	7/16	3/16	21	0.040 (0.010–0.161)
B	6/16	4/16	3/16	4/16	7	—[c] —[c]
B	12/16	4/16	0/16	1/16	10	0.185 (0.072–0.485)
B	11/16	6/16	0/16	0/16	14	0.173 (0.064–0.471)

[a] Mice were immunized ip on days 0 and 3.
[b] Survivors/total.
[c] Could not be calculated from data.

TABLE 2. *Effect of different challenge levels on the ED_{50} of the vaccine*

LD_{50} of challenge virus	Vaccine dose[a] (ml in 0.2 ml)				ED_{50} ± 1 SD (ml)
	0.2	0.04	0.008	0.0016	
3,200,000	10/16[b]	8/16	2/15	1/16	0.143 (0.089–0.230)
320,000	10/16	6/16	2/14	0/14	0.185 (0.115–0.300)
32,000	11/16	8/14	2/15	2/15	0.120 (0.079–0.181)
3,200	11/15	8/15	1/16	0/15	0.079 (0.060–0.105)
320	10/16	7/16	1/16	0/14	0.092 (0.065–0.129)
32	12/16	6/15	2/16	1/14	0.130 (0.091–0.187)

[a] Given ip on days 0 and 3.
[b] Survivors/total.

one standard deviation limits of the ED_{50} values all overlap. Moreover, these values have a two- to threefold variation which subsequent data will indicate is smaller than the sensitivity of the assay. Therefore all subsequent mouse assays were conducted with 100 to 1,000 LD_{50} of virulent virus, although this was not a critical factor.

Sensitivity of the mouse assay. To evaluate the sensitivity of the assay, ED_{50} values were determined for the original vaccine and for three dilutions of this vaccine (1:2, 1:4, and 1:8).

When the ED_{50} values of vaccines B and C (Table 3) were divided by two and four, respectively, to convert them to the same basis as vaccine A, the ED_{50} values (0.120 for vaccine B and 0.138 for vaccine C) were within the limits of ±2 SD of vaccine A. Therefore, the ED_{50} values were comparable, and the response of the mice did not significantly change with dilution of the vaccine. However, when the vaccines were treated as separate entities and the ±2 SD (ml) values were compared, a twofold difference in potency would not be detected; but there is a significant difference at the fourfold level. Thus, for a vaccine to be considered more effective than a reference vaccine, its ED_{50} value would have to be four times greater than the ED_{50} value of the reference vaccine in a mouse potency assay.

Comparison of mouse and guinea pig assays. Since guinea pigs have been used as the standard animal for WEE vaccine potency testing, the responses of mice and guinea pigs to immunization with the same dilutions of five different vaccines were compared (Table 4). Although, the guinea pigs gave lower ED_{50} values than did the mice, the guinea pig ED_{50} values also had greater confidence limits than the mouse values. This may be a reflection of the smaller numbers of animals used per dilution in the guinea pig test (10 as compared to 16 in the mouse assay).

By comparison of the confidence limits, vaccine C was less potent than vaccine D in both mice and guinea pigs, whereas vaccines E, F, and G could not be differentiated from each other. However, vaccines E, F, and G could be differentiated from D. Therefore, the ranking of the vaccines in both mouse and guinea pig assays was identical: C, then D, and then a group composed of E, F, and G.

DISCUSSION

The final mouse potency assay that was developed consisted of ip vaccination with 0.2-ml

TABLE 3. *Sensitivity of the mouse assay as determined with dilutions of vaccine*

Vaccine[a]	Vaccine dose[b] (ml in 0.2 ml)			ED_{50} ± 2 SD (ml)
	0.2	0.04	0.008	
A	10/16[c]	7/16	1/16	0.173 (0.057–0.522)
B	9/15	6/16	1/15	0.239 (0.074–0.774)
C	6/16	1/16	0/15	0.553 (0.182–1.692)
D	1/15	1/15	0/16	—[d]

[a] Vaccine A was undiluted, vaccine B was A diluted one-half, vaccine C was A diluted one-fourth, and vaccine D was A diluted one eighth.
[b] Mice were vaccinated ip on days 0 and 3 and challenged IC with 320 LD_{50} of virulent virus on day 14.
[c] Survivors/total.
[d] Could not be calculated from experimental results.

TABLE 4. *Comparison of ED_{50} values derived from guinea pig and mouse potency assays*

Vaccine	Guinea pigs		Mice	
	ED_{50} ± 2 SD (ml)	Relative potency[a]	ED_{50} ± 2 SD (ml)	Relative potency[a]
C	0.667 (0.153–2.861)	1	0.235 (0.078–0.719)	1
D	0.059 (0.011–0.317)	11	0.084 (0.032–0.222)	3
E	0.005 (0.002–0.016)	133	0.019 (0.007–0.052)	12
F	0.003 (0.001–0.013)	222	0.019 (0.006–0.057)	12
G	0.002 (0.001–0.012)	334	0.021 (0.007–0.068)	11

[a] ED_{50} of vaccine C divided by the ED_{50} of the vaccine being tested.

doses of serial fivefold dilutions of vaccine on days 0 and 3 and subsequent ic challenge containing 100 to 1,000 LD_{50} of virulent virus on day 14. The assays were terminated and statistically evaluated at 14 days postchallenge. Dilutions of vaccine were chosen to include 0 and 100% mortality after challenge with virulent virus. Obviously, higher dilutions would have to be used if a more potent vaccine were being assayed.

The similarity of the ED_{50} values when the vaccinated mice were challenged with $\log_{10}$ dilutions of virulent virus was comparable to that found with formalinized Rift Valley Fever vaccine (1). In both cases, the critical factor was the amount of antigen in the immunizing dose rather than the LD_{50} of virulent virus in the challenge dose. This enables valid potency and stability comparisons to be made even if the challenge dose varies greatly.

When the same vaccines were compared in mice and guinea pigs by using parallel assays, there were no differences in the rankings, which indicates that comparable data could be derived from either assay.

The mouse potency assay for WEE vaccine is advantageous in several ways. The assay is completed in 28 days, which is 7 days less than the guinea pig or hamster assays. In addition, larger numbers of mice can be housed in a given area, thus increasing the number of assays that may be conducted and the number of mice that can be used per assay. This would increase the statistical validity of the assay. Finally, animal costs are reduced by 85% as compared to the other assay systems.

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