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23 June 1972

APPLIED MICROBIOLOGY, Oct. 1972, p. 604-608

Vol. 24, No. 4
Printed in U.S.A.

Early Protection in Hamsters Immunized with Attenuated Venezuelan Equine Encephalomyelitis Vaccine

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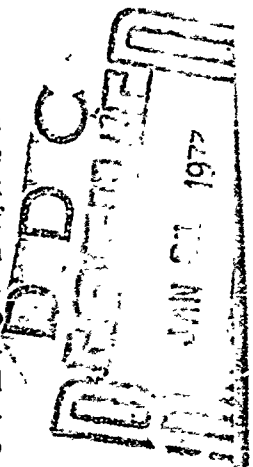
Received for publication 23 June 1972



The rapid onset and persistence of homologous and heterologous protection induced by attenuated Venezuelan equine encephalomyelitis (VEE) vaccine (TC-83) were studied in the hamster, by using challenge response as the index of protection. At 8 hr postvaccination with 10^3 median immunizing doses of TC-83 vaccine, 15 to 20% of animals were protected against challenge with VEE virus as well as Eastern and Western equine encephalomyelitis viruses. The percentage of protection increased with time postvaccination until 80 to 90% homologous and heterologous protection was achieved by 18 hr postvaccination. Temporal studies indicated that early protection (days 1 to 6) correlated with vaccine viremia, and that the percentage of protection against heterologous challenge decreased with the cessation of viremia. Data are presented to indicate that the early protection phenomenon is one of interference, since little or no replication of a challenge virus occurred when it was administered during the vaccine viremia stage.

In a previous publication (5) we reported that, in the hamster, attenuated Venezuelan equine encephalomyelitis (VEE) vaccine (TC-83) produced excellent homologous protection as well as 59 and 37% protection against Eastern and Western equine encephalomyelitis (EEE, WEE) virus challenge, respectively. However, in those studies all challenges were made 21 to 30 days postvaccination. Because of the extensive VEE epizootics in Central America (R. O. Spertzel and R. W. McKinney, *in press*) and Mexico (R. O. Spertzel and R. W. McKinney, *in press*) in 1969 to 1971, and the subsequent outbreak of the disease in the United States (R. O. Spertzel, *in press*) in the summer of 1971, the studies described herein were conducted to determine if the TC-83 vaccine was capable of inducing rapid protection. The significance of this is apparent when one considers: (i) the speed with which VEE spreads in an equine population, and (ii) the fact that immunization is often performed in areas where active cases are occurring. Also, it was deemed desirable to determine whether the vaccine would provide significant, rapid cross-protection against EEE and WEE, the two other equine encephalomyelitides endemic in those areas of the United States in which VEE outbreaks could be expected to occur. Finally, the possible mechanism(s) of early protection was investigated.

MATERIALS AND METHODS

Challenge viruses. California strain WEE virus (3), Cambridge strain EEE virus (3), and Trinidad strain VEE virus (12) were employed as challenge viruses. These strains were lethal to high titers, i.e., 10^7 to 10^{10} median lethal doses (LD_{50}) per milliliter, when given intraperitoneally (ip) to the hamster.

Virus titrations. All challenge viruses were titrated via the ip route in Lakeview strain golden Syrian hamsters (85 to 95 g) obtained from the Lakeview Hamster Colony, Newfield, N.J. Groups of six hamsters were inoculated with 0.5 ml of $\log_{10}$ dilutions of virus in cold phosphate-buffered saline, *p*H 7.2, containing 1% normal rabbit serum (PBS). For viremia determinations with attenuated VEE (TC-83) vaccine, blood specimens were diluted in cold PBS. Groups of six 1- to 3-day-old mice (CD-1 strain; Charles River Mouse Farm, Wilmington, Mass.) were then inoculated intracerebrally (ic) with 0.03 ml of $\log_{10}$ dilutions. Titrations end points in hamsters and suckling mice were determined by the method of Reed and Muench (13) and are expressed as LD_{50} per milliliter.

Vaccination and challenge. The attenuated VEE (TC-83) vaccine has been described (2, 10). In all experiments, hamsters were inoculated ip with 10^3 median immunizing doses (ID_{50}) of the vaccine. Challenges were made via the ip route at the indi-

cated times using 10^3 hamster LD_{50} of a given challenge virus. Normal hamsters used as challenge controls for all experiments consistently showed a 98 to 100% death rate with the dose of challenge virus employed.

Serology. Randomly selected hamsters were bled at the specified periods postvaccination or postchallenge. Sera were stored at −20 C until tested for hemagglutination-inhibiting (HI) antibody, by the method of Clarke and Casals (4), as modified by French and McKinney (8).

RESULTS

Temporal studies. Initial studies were aimed at determining: (i) the degree of homologous and heterologous protection induced by the vaccine during the first 10 days postvaccination, and (ii) the presence of circulating HI antibody or TC-83 virus, or both, during this period. Table 1 is a compilation of data from several experiments in which hamsters were administered TC-83 vaccine on day 0. On days 1 through 10 postvaccination, representative numbers of hamsters were exsanguinated for vaccine viremia and HI antibody determinations, while other randomly selected hamsters were challenged with virulent VEE, EEE, or WEE viruses. Vaccine viremia appeared as early as day 1 postvaccination and persisted through day 5 in the majority of hamsters; however, viremia was demonstrated in some animals as late as day 7. Termination of the viremic stage generally coincided with the appearance of HI antibody to TC-83 virus. HI antibody to TC-83 virus was detected in individual animals as early as day 5, whereas in others antibody did not appear until day 8. A high degree of homologous and heterologous protection was afforded those animals challenged during the vaccine viremia stage, viz. days 1 through 6, after which heterologous protection gradually decreased. Only 0 to 2% of nonvaccinated, control animals survived challenge.

TABLE 1. *Response of hamsters vaccinated with TC-83 vaccine and challenged with VEE, EEE, or WEE viruses*[a]

Day post-vaccination	VEE HI antibody	Vaccine viremia	Percent survivors (no./total) after ip challenge with 10^3 LD_{50}[b]		
			VEE	EEE	WEE
1	−[c]	+/−[d]	92 (23/25)	87 (26/30)	93 (37/40)
2	−	+	90 (18/20)	90 (27/30)	77 (27/35)
3	−	+	92 (46/50)	79 (123/155)	83 (132/160)
4	−	+	92 (46/50)	77 (46/60)	77 (50/65)
5	+/−	+/−	100 (20/20)	87 (26/30)	88 (30/34)
6	+/−	+/−	100 (20/20)	77 (23/30)	97 (34/35)
7	+/−	+/−	85 (17/20)	59 (17/29)	59 (20/34)
8	+	−	100 (15/15)	72 (18/25)	63 (19/30)
9	+	−	93 (14/15)	40 (10/25)	63 (19/30)
10	+	−	100 (15/15)	48 (12/25)	57 (17/30)

[a] Data compiled from results of several experiments. Abbreviations: VEE, EEE, WEE—Venezuelan, Eastern, and Western equine encephalomyelitis, respectively; HI, hemagglutination-inhibiting.

[b] Nonvaccinated (control) animals exhibited 0 to 2% survival after challenge.

[c] A titer of ≥1:10 considered positive.

[d] See Results.

TABLE 2. *Development of viremia and HI antibody in hamsters vaccinated with TC-83 vaccine*

Day postvaccination	Vaccine viremia[a]		VEE HI antibody titer[b]
	Percent (no./total)	Log_{10} mean (range)	
1	100 (10/10)	2.2 (1.5-2.8)	<10
2	100 (10/10)	3.7 (1.3-5.7)	<10
3	100 (10/10)	5.7 (1.9-6.7)	<10
4	100 (10/10)	2.9 (1.0-4.7)	<10
5	90 (9/10)	1.4 (0.6-2.4)	≤10
6	20 (2/10)	2.1 (1.5-3.3)	20
7	30 (3/10)	1.3 (1.3-2.0)	40
8	0 (0/10)		20
9	0 (0/10)		160
10	0 (0/10)		80

[a] Ten different animals bled each day; titers expressed as log_{10} suckling mouse ic LD_{50}/ml.

[b] Expressed as reciprocal of titer; pooled sera from two hamsters tested on each day postvaccination. For abbreviations, see footnote to Table 1.

A further study was made to quantify the viremia and HI antibody levels observed during the first 10 days postvaccination. Hamsters were inoculated with TC-83 vaccine and were then randomly selected and exsanguinated for determination of viremia (10 hamsters/day) or HI antibody (2 hamsters/day). As shown in Table 2, all hamsters examined on days 1 through 4 were viremic, with peak mean levels occurring on day 3. All animals tested for viremia after day 7 were negative. HI antibody was not detected until day 5, after which there was the expected increase in titer with time postvaccination.

Mechanism of early protection. In view of the high degree of homologous and heterologous protection observed at 24 hr postvaccination, an additional study was performed to determine the protection afforded at earlier periods postvaccination and to relate this to vaccine viremia. Subsequent to vaccination, hamsters were either bled for viremia determinations or challenged with VEE, EEE, or

WEE viruses. Table 3 is a summary of the results of such a study performed at 8, 12, and 18 hr postvaccination. The importance of vaccine viremia in the early protection stage is reflected in the responses to the challenge viruses. As the percentage of viremic animals increased with time postvaccination, so also did protection against homologous and heterologous challenge. The results observed at 18 hr postvaccination are comparable to those seen previously at 24 hr postvaccination (cf. Table 1).

To gain further insight into the mechanism of early protection, we studied those hamsters that survived a challenge administered during the period of highest vaccine virema, i.e., days 1 through 4 postvaccination. In separate experiments, groups of hamsters were vaccinated on day 0 and then challenged with VEE, EEE, or WEE viruses on days 1, 2, 3, or 4. Control animals received only TC-83 vaccine on day 0. On day 17 postvaccination, representative numbers of hamsters that survived challenge with virulent VEE, EEE, or WEE viruses as well as hamsters that had received only TC-83 vaccine on day 0 were exsanguinated, and HI antibody titers were determined. The remaining animals in each survival group were rechallenged with the same virus that had been administered on days 1, 2, 3, or 4. As controls, hamsters that had received only TC-83 vaccine on day 0 and normal hamsters of the same age were also challenged. On day 34, randomly selected surviving animals from all groups were bled for HI antibody determinations. Results of these studies are summarized in Table 4. As indicated, there was virtually no difference in day 17 survival rates between those animals that had received a previous homologous challenge and those that received only TC-83 vaccine on day 0. Since significant replication of the original (day 1 to 4) challenge virus should have increased the ability of these animals to withstand rechallenge, one can assume that little or no replication occurred. This apparent lack of replication by the original challenge virus is verified by the HI titers of animals at the time of rechallenge on day 17. Comparable titers were seen in the challenged (primary) and control groups for each of the three viruses studied. These data are suggestive of an interference phenomenon mediated directly or indirectly by the TC-83 vaccine. Supporting this belief is the indirect evidence of the ability of EEE and WEE viruses to replicate in the absence of interfering virus, even in animals previously protected by inoculation with TC-83 vaccine. As shown in the results of HI tests performed with sera obtained from hamsters on day 34 (i.e., 17 days after rechallenge), marked increases of a similar magnitude in HI titers against EEE and WEE viruses occurred with both the previously challenged and control groups.

TABLE 3. *Early response of hamsters vaccinated with TC-83 vaccine and challenged with VEE, EEE, or WEE viruses*

Hr postvaccination	Percent vaccine viremia (no./total)	Percent survivors (no./total) after ip challenge with 10^3 LD_{50}[a]		
		VEE	EEE	WEE
8	20 (2/10)	15 (3/20)	15 (3/20)	20 (4/20)
12	50 (5/10)	30 (6/20)	25 (5/20)	25 (5/20)
18	100 (5/5)	90 (18/20)	80 (16/20)	90 (18/20)

[a] All challenge control hamsters died at each time period with each challenge virus. For abbreviations, see footnote to Table 1.

TABLE 4. *Response to homologous rechallenge in TC-83 vaccinated hamsters that survived a primary challenge with VEE, EEE, or WEE viruses*[a]

Primary challenge on day 1 to 4 postvaccination[b]	Percent (no./total) surviving rechallenge on day 17 with 10^3 LD_{50}	Homologous mean HI titer[c] (range)	
		Day 17	Day 34
VEE	98 (49/50)	280 (160-320)	240 (80-320)
Control[d]	100 (27/27)	480 (160-1280)	486 (40-1280)
EEE	39 (47/120)	17 (10-20)	1044 (40-1280)
Control[d]	35 (15/43)	19 (<10-40)	820 (40-2560)
WEE	42 (52/125)	21 (<10-20)	592 (160-1280)
Control[d]	44 (19/43)	25 (<10-40)	520 (160-1280)

[a] Data compiled from results of several experiments. For abbreviations, see footnote to Table 1.

[b] Received TC-83 vaccine on day 0, and challenge virus indicated on day 1, 2, 3, or 4 postvaccination.

[c] Reciprocal of geometric mean and range determined using pooled sera of two hamsters each; minimum of five pools tested against appropriate antigen (VEE, EEE, or WEE).

[d] Received TC-83 vaccine on day 0, but were not challenged until day 17.

Effects of vaccination after or at time of challenge. As a result of our demonstration of the rapid onset of viremia in animals administered TC-83 vaccine (Table 3), it was of practical significance to determine whether vacci-

nation would also be efficacious if carried out simultaneously, or after challenge with VEE, EEE, or WEE viruses. In a limited study, hamsters were administered TC-83 vaccine 48 or 24 hr after challenge as well as simultaneously with the challenge viruses. As shown in Table 5, little or no protection was afforded animals vaccinated after challenge. The degree of protection seen against WEE virus challenge is perhaps due to the slower invasiveness of this virus in the hamster, although no studies to confirm this were conducted.

TABLE 5. *Effect of vaccination with TC-83 vaccine following inoculation of hamsters with virulent VEE, EEE, or WEE viruses*

Virulent virus[a]	Survivors (no./total) by hr of challenge preimmunization:[b]		
	48 hr	24 hr	0 (simultaneous)
VEE	0/5	0/5	0/5
EEE	0/5	0/5	0/5
WEE	0/5	1/5	3/5

[a] Challenge was 10^3 LD_{50}, ip at 48 hr or 24 hr prior to or simultaneously with vaccine. For abbreviations, see footnote to Table 1.

[b] All hamsters received 10^3 ID_{50} of TC-83 vaccine, ip at time 0.

DISCUSSION

Although extensive immunization programs and some studies have been conducted with TC-83 vaccine in man (1, 7, 11; P. J. Bartelloni, *personal communication*) and animals (5, 6, 14), no information has been available regarding the ability of the vaccine to induce rapid homologous and heterologous protection.

In the present study we have demonstrated that protection in the hamster starts as early as 8 hr postvaccination, and that early protection closely parallels the viremia resulting from infection with the vaccine virus. Homologous and heterologous protection was greatest during the period of maximum vaccine viremia, i.e. days 1 to 6. The persistence of heterologous protection after this period was not unexpected in view of the results of an earlier study in which we demonstrated the ability of TC-83 vaccine to elicit 59 and 37% protection against EEE and WEE virus challenges, respectively, 21 to 30 days postvaccination (5).

Our data on early protection strongly suggest that an interference phenomenon is responsible for the degree of protection observed. In this case the presence of a replicating, or at least, circulating virus (i.e., TC-83 vaccine) prevents superinfection of a host animal with other viruses. The failure of the challenge viruses to replicate to any appreciable extent when administered during the vaccine viremia stage was indirectly demonstrated in two ways: (i) hamsters which survived such a challenge did not show increased resistance to a rechallenge administered after the vaccine viremia stage, and (ii) the HI antibody responses of hamsters that were vaccinated and challenged were similar to those of hamsters that were only vaccinated. Evidence of the nonspecific nature of the early protection is provided by results of experiments in which it was shown that EEE and WEE viruses do, indeed, replicate in the absence of interfering virus, namely in the same animals that had been previously protected by inoculation with TC-83 vaccine. Here, death or marked increases in HI antibody titers to EEE and WEE viruses were similar in both test and vaccine control animals. It should be noted that the failure of late challenge with virulent VEE virus to increase the homologous antibody titer is in agreement with the results of our earlier study (5). Our findings, in general, are in agreement with those of Hearn and Rainey (9), who showed that a variety of laboratory animals were protected against homologous and heterologous challenge as early as 24 hr postvaccination with another attenuated strain of VEE virus. In our study, no attempt was made to determine whether the observed interference was interferon-mediated.

In terms of field use of the attenuated VEE vaccine in equines, the results of these early protection studies are of major importance. For example, Spertzel and McKinney reported that during the 1969 Central American epizootic of VEE, all equine cases of VEE stopped 7 to 10 days after vaccination, even in areas experiencing active cases of VEE in nonimmunized animals (R. O. Spertzel and R. W. McKinney, *in press*). Similar results were reported by Eddy et al. (6). If one assumes that the majority of thos equines which died or exhibited clinical illness during the 7- to 10-day period were infected prior to or shortly after vaccination, then one can also assume that protection occurs in equines earlier than 7 days postvaccination. These assumptions are supported by Walton's analysis of the VEE outbreaks in Nicaragua and Costa Rica, which indicates that the vaccine will protect equines within 3 days even in the presence of concurrent equine VEE infections in a given area (T. E. Walton, *in press*).

Based on the studies reported here, one might also expect rapid, solid protection of equines against naturally occurring WEE and EEE during the earlier periods postvaccination. Indeed, a decrease in WEE cases concurrent with the VEE vaccination campaign during the summer of 1971 in southern California was noted (W. C. Reeves, *personal communication*). Thus, the attenuated VEE vaccine can be expected to elicit rapid protection not only against VEE virus, but also against the other two major equine encephalitides. Our previous studies indicate that such heterologous protection cannot be expected from an inactivated vaccine prepared from VEE virus (5).

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