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Protective Efficacies of Live Attenuated and Formaldehyde-Inactivated Venezuelan Equine Encephalitis Virus Vaccines Against Aerosol Challenge in Hamsters

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Although two investigational vaccines are used to immunize humans against Venezuelan equine encephalomyelitis virus, neither had previously been tested for protective efficacy against aerosol exposure. Live attenuated vaccine (TC-83) protected all hamsters challenged by either aerosol or subcutaneous routes with 4.7 to 5.2 $\log_{10}$ PFU of virulent Venezuelan equine encephalomyelitis virus. Formaldehyde-inactivated vaccine (C-84) failed to protect against aerosol challenge but did protect against subcutaneous challenge. Protection elicited by TC-83 vaccine did not depend solely on serum-neutralizing antibody. These studies suggest that TC-83 vaccine is preferable to C-84 vaccine for protecting laboratory workers at risk to aerosol exposure.

Two different investigational vaccines approved for limited human use are available for immunization against Venezuelan equine encephalomyelitis (VEE) virus. One of these vaccines, a live attenuated strain (TC-83), has been used since 1961 to protect laboratory and field workers who are at risk of infection with virulent strains of this virus (20, 21). Although extensive experience with TC-83 vaccine in more than 6,000 individuals suggested excellent protective efficacy and safety (20), concerns arose about the use of live attenuated vaccines in general and TC-83 in particular. Its reported reactogenicity (1, 21), potential to revert in virulence (4) and possibly to enter a mosquito-mammal cycle (20), and its teratogenic potential (19) suggested the need to develop an inactivated vaccine. This second vaccine, C-84 (6), is a formaldehyde-inactivated preparation of TC-83. The serum antibody titers induced in humans by C-84 (12) were generally lower than those induced by TC-83 (5) but were considered adequate for protection, especially since uniform protection was induced by C-84 in standardized laboratory animal tests. All available protective efficacy data for both VEE vaccines are based on survival after subcutaneous (s.c.) or intraperitoneal (i.p.) challenge by injection, although VEE virus is known to be infectious by the aerosol route (9, 10, 14) and is believed to have caused laboratory infections by aerosol (28). Therefore, comparative assessments of the efficacies of the two vaccines against aerosol exposure were needed.

Hamsters were selected for immunization and challenge based on extensive experience in previous vaccine efficacy tests (7, 8), the pronounced sensitivity of hamsters to VEE challenge by any route (7), and the bimodal nature of the acute disease involving primarily the reticuloendothelial system but also the central nervous system (11, 16, 17, 29). Groups of adult male golden Syrian hamsters, weighing 90 to 110 g, were inoculated with either TC-83 or C-84 vaccine. Both vaccines were freshly reconstituted and inoculated i.p. without further dilution in 0.5-ml volumes. Hamsters inoculated with live attenuated vaccine strain TC-83 (NDBR-102, lot 4, run 2; National Drug Co., Swiftwater, Pa.) received 3.5 $\log_{10}$ PFU per 0.5 ml and were not further manipulated until challenged. Hamsters receiving inactivated VEE vaccine (MNLBR-109, lot C-84-1; Merrell-National Laboratories, Swiftwater, Pa.) were inoculated twice at a 14-day interval and then rested until challenged. Challenge VEE virus (Cx) was prototype Trinidad donkey strain 1-2AC-8 which had been passaged nine times in embryonated chicken eggs, four times in chick embryo cell cultures, and one additional time in suckling mouse brains, as reported previously (15). For aerosol exposure and s.c. inoculation, Cx was diluted in Eagle minimal essential medium containing Earle salts supplemented with nonessential amino acids, glutamine, and 10% inactivated fetal bovine serum. For aerosol challenge, unanesthetized hamsters were exposed for 10 min, using a dynamic system consisting of a modified Henderson tube with a Collison generator (18) at 22°C. Infectious aerosol exposure doses were calculated by standard procedures (13). For s.c. challenge, Cx was diluted and inoculated in 0.5-ml volumes. All hamsters were challenged 45 days after the first vaccine dose.

All hamsters previously inoculated with the live attenuated TC-83 strain resisted the virulent Cx strain challenge in both high- and low-dose exposures given via the aerosol or s.c. route (Table 1). In contrast, hamsters inoculated with the inactivated C-84 vaccine regimen were less uniformly protected. All 14 C-84-inoculated hamsters died after challenge with 4.7 $\log_{10}$ PFU by the aerosol route. Of 14, 3 (21%) died after low-dose (2.5 $\log_{10}$ PFU) aerosol exposure, as did 2 of 14 and 1 of 14 exposed to high- and low-dose s.c. challenge, respectively.

The differences observed in protection against aerosol challenge could depend on qualitative differences in humoral or cellular immune responses elicited or on quantitative differences. For 10 unchallenged hamsters inoculated with TC-83 vaccine and tested on day 45, the geometric mean neturalizing antibody titer, expressed as the serum dilution neutralizing 80% of plaques in a serum dilution test (PRN-80), was 1:257. For C-84-vaccinated hamsters, the PRN-80 was 1:8. To determine whether this difference in serum-neutralizing antibody titers could account for the observed differences in sensitivity to high-dose aerosol challenge, 30 hamsters were passively immunized i.p. with 5 ml of hyperimmune hamster serum to attain an average PRN-80 titer of

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TABLE 1. Protective efficacy in hamsters of live attenuated (TC-83) and formaldehyde-inactivated (C-84) VEE vaccines versus aerosol (A) and s.c. challenge with the Trinidad strain of VEE virus

Vaccine	Challenge		Dead/total (%)	MDTD[a] (range)
	Route	Log_{10} PFU		
TC-83	A	2.5	0/14 (0)	
	A	4.7	0/14 (0)	
	s.c.	2.9	0/14 (0)	
	s.c.	5.2	0/14 (0)	
C-84	A	2.5	3/14 (21)	7.0 (4–10)
	A	4.7	14/14 (100)	4.0 (4)
	s.c.	2.9	1/14 (7)	4.0 (4)
	s.c.	5.2	2/14 (14)	5.5 (5–6)
None	A	2.5	10/10 (100)	4.8 (4–6)
	A	4.7	10/10 (100)	3.7 (3–4)
	s.c.	2.9	10/10 (100)	4.1 (3–6)
	s.c.	5.2	10/10 (100)	3.7 (3–4)

[a] MDTD, Geometric mean days to death.

1:209. These passively immunized hamsters resisted s.c. challenge (5.1 log_{10} PFU) but were uniformly sensitive to aerosol exposure (Table 2). The resistance to aerosol exposure elicited by TC-83 vaccine thus depends on factors other than serum-neutralizing antibody titers alone. Further support for this concept was obtained in recent studies in which it was shown that aerosol administration of measles vaccine was able to overcome resistance to s.c. infection posed by maternal antibodies present in young children (25, 26).

The mechanism by which aerosolized Cx kills C-84-inoculated or passively immunized hamsters is important to determine. Local immunity, in the form of secretory immunoglobulin A, is known to be important in resistance to respiratory viruses such as respiratory syncytial virus (22), influenza virus (27), and rhinoviruses (24). Live replicating antigen is superior to inactivated antigen in eliciting local immunity in other systems (23, 25, 26) and may be a factor in the superior resistance elicited by TC-83. Virulent VEE is often considered to be a neurotropic virus, and it further exhibits significant infectivity via the respiratory tract. The potential of VEE virus to invade the central nervous system via the cribriform plate has been documented for nonhuman primates (9, 10), and in hamsters VEE virus has been shown to invade the olfactory bulb (11). Local immunity may prevent the invasion of olfactory neurons at the level of the cribriform plate. Sequential sacrifice studies to assess infectivity and tissue pathology in C-84-vaccinated, aerosol-challenged hamsters may define the mechanism by which VEE virus can reach the central nervous system despite serum-neutralizing antibodies.

Although studies are under way to determine mechanisms of disease and of protection, the observed failure of C-84 vaccine to protect against aerosol challenge represents a clear danger signal to laboratory workers immunized with this vaccine who may have a false sense of security. Although these results in hamsters may not be directly applicable to humans, these studies also raise some doubts about the protective efficacies against aerosol challenge of other inactivated vaccines. Specifically, the two commonly used alphavirus vaccines, those used against western and eastern equine encephalomyelitis viruses (2, 3), are of interest since attenuated vaccine strains suitable for human immunization have not been developed for these two viruses. For VEE virus, however, a choice of vaccines does exist. The results of this study suggest that immunization with the live attenuated strain is clearly preferable for protecting laboratory workers and other individuals at risk to aerosol exposure.

TABLE 2. Protective efficacy of passively transferred immune serum for hamsters challenged with VEE virus

Immune serum[a]	Challenge		Dead/total	MDTD[b] (range)
	Route	Dose (log_{10} PFU)		
5 ml	A[c]	5.2	15/15	4.9 (4–7)
5 ml	s.c.	5.1	0/15	
None	A	5.2	4/4	3.0 (3–3)
None	s.c.	5.1	4/4	3.2 (3–4)

[a] Hyperimmune hamster serum, inoculated i.p. The average PRN-80 was 1:209.
[b] MDTD, Geometric mean days to death.
[c] A, Aerosol.

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