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Isolation of Two Plaque Mutants of Western Equine Encephalitis Virus Differing in Virulence for Mice

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Two plaque mutants were isolated from tissue cultures infected persistently with Western equine encephalitis virus. A large plaque mutant proved to be markedly avirulent for mice.

The mutants of Western equine encephalitis (WEE) virus have been reported concerning plaque type (2–4) and virulence for mice (1). Less attention has been paid thus far to the genetic markers to differentiate strains of attenuated and virulent WEE virus. This paper concerns the isolation and initial characterization of two attenuated mutants of WEE virus.

The parent strain of WEE virus which was used for this study had been passed in adult mouse brain many times in this laboratory (5). Before the experiment, cloning of the virus was performed by three successive passages of single plaques on chick embryo fibroblast (CEF) cells. CEF cells were prepared by trypsinization of 10-day-old eviscerated embryos. The growth medium for cells consisted of Eagles minimum essential medium, 5% bovine serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. A 5-ml amount of the suspended cells was placed in 60-ml glass bottles. After incubation for 24 hr, at 37 C, the monolayer cultures were infected with 0.2 ml of the virus suspensions and overlaid after 60 min of incubation. The agar overlay medium consisted of the growth medium described above with neutral red (1:90,000) and 1.4% agar (Difco). For the experiment on persistent infection of the WEE virus, Frukto cells (6) were used which derived from a mouse sarcoma. This cell line was grown in the same medium for CEF cells.

During the course of investigation into the multiplication of WEE virus in Frukto cells, it was noted that in most cases the cytopathic effect exerted by the virus was incomplete, and a carrier-state population was estabished. Such infected population was quite stable, and in the culture fluid active virus was consistently present [approximately 10^3 to 10^4 plaque-forming units (PFU)/ml]. At 4 months after initial infection to Frukto cells, two plaques different in size to those of the parent strain were observed at low rate (Fig. 1). Both mutants were cloned by three successive passages of single plaques on CEF cells.

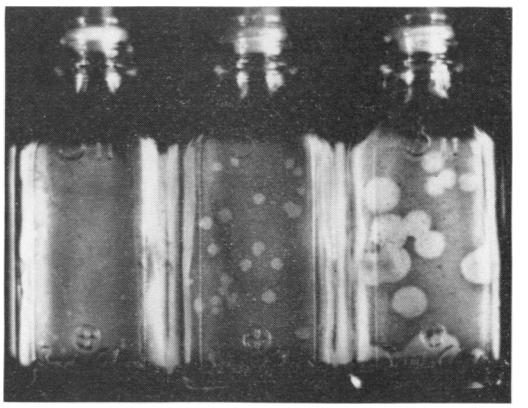

FIG. 1. *Plaques produced with the parent strain of WEE virus in CEF cells (left); large plaques formed with the W232 (middle) and the W433 mutant (right). Plaques were restained with neutral red at 5 days after inoculation.*

In three experiments, diameters of 100 plaques of each strain were measured by a pair of calipers 5 days after inoculation. Calculations were made of the mean and standard deviation of plaque size for each strain (Table 1). The plaque size of W232 and W433 mutants remained unchanged after successive passages in suckling and adult mouse brain, respectively.

The parent strain was highly virulent for the Swiss ddC line of 1-week-old adult mice and 1-day-old suckling mice. Mouse brain emulsion

TABLE 1. *Comparison of plaque diameters in chick embryo fibroblast (CEF) cells and virulence for mice of western equine encephalitis (WEE) virus mutants*

WEE virus strain	Plaque diameter	Log_{10} PFU/ml	Log_{10} LD_{50}/ml	
		CEF cells	Adult mice	Suckling mice
Parent grown in adult mice.....	1.2 ± 0.4 mm[a]	9.0[b]	8.6[b] (1.5)[c]	8.7[b] (1.5)[c]
W232 grown in CEF cells.....	2.9 ± 0.7 mm	7.3	<1.7	7.4 (4.0)
W433 grown in adult mice.....	5.8 ± 1.9 mm	8.5	7.9 (5.0)	8.2 (3.5)

[a] Mean diameter and standard deviation were calculated from 100 plaques of each strain.

[b] Comparative titration was done simultaneously in CEF cells (0.2 ml), in adult mice with intracerebral inoculation (0.02 ml), and in suckling mice with subcutaneous inoculation (0.02 ml) for each strain.

[c] Figures in parentheses indicate the mean death time (day) when mice were inoculated with 100 PFU of each strain.

(10%) which was infected with the parent strain obtained approximately $10^{8.6}$ LD_{50}/ml when injected intracerebrally into adult mice, or $10^{8.7}$ LD_{50}/ml when inoculated subcutaneously into suckling mice. The virulence of the mutants for mice differed markedly from that of the parent virus (Table 1). Both the incubation period and the interval between the onset of paralysis and death were prolonged by 2 to 3 days after inoculation of the W433 mutant. Following inoculation of the W232 mutant, no death was scored in adult mice. The adult mice inoculated intracerebrally with more than 10 PFU of the W232 strain were completely immune 3 weeks later to intracerebral challenge with 100 LD_{50} of the parent strain or with the WEE virus strain obtained from the National Institute of Health in Tokyo.

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