

## Neurovirulence and Induction of Hydrocephalus with Parental, Mutant, and Revertant Strains of Measles Virus

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The relationship between neurovirulence and induction of hydrocephalus was investigated for a measles virus temperature-sensitive mutant and its revertant. The revertant regained the neurovirulence of the parental strain. At appropriate doses the parental, mutant, and revertant strains induced hydrocephalus.

A temperature-sensitive mutant of measles virus (ts G) with decreased neurovirulence was reported to induce hydrocephalus in suckling hamsters (6). To further investigate the relationship between this temperature-sensitive mutation and the alteration of neuropathogenic properties, we isolated a non-temperature-sensitive revertant of ts G. For the mutant, revertant, and parental strains, we determined (i) neurovirulence and (ii) ability to induce hydrocephalus. Our results indicate that reversion of the ts marker is associated with complete reversion to virulence. We also found that at sublethal doses the revertant and parental strains can cause hydrocephalus. Thus, induction of hydrocephalus is not a unique property of the ts G mutant. We suggest that the decreased neurovirulence of this mutant accounts for its ability to induce hydrocephalus with high frequency.

The isolation and characterization of temperature-sensitive mutants of measles virus were previously described (4). The derivation of the parental strain (CC) was also previously reported (5). In the present study, a plaque-purified virus stock (designated G-3) of ts G (3) was used. Virus stocks were prepared and virus assays were carried out in BSC-1 cells (originally obtained from R. Dulbecco).

A ts<sup>+</sup> revertant of ts G was isolated by plating a low dilution of the mutant at 39 C and picking individual plaques. A virus stock grown from one such plaque isolate was used in these experiments. The ratios of plaquing efficiencies at 39 and 33.5 C of this revertant (designated G-9) and the parental strain were identical (Table 1). However, G-9 differed from the parental strain by at least one other property (McKimm and Rapp, personal communication); therefore, G-9 was clearly a ts<sup>+</sup> revertant and not a wild-type contaminant. When inoculated intracra-

nially into newborn hamsters all three strains produced an encephalitis. However, G-9 and CC were much more neurovirulent than G-3 (Table 1 and Fig. 1A). The fact that G-9 was

TABLE 1. Neurovirulence of measles virus strains in newborn hamsters

Strain	Phenotype	Efficiency of plaquing (39/33.5 C)	LD <sub>50</sub> (PFU) <sup>a</sup>
CC	Parent	10 <sup>-0.2</sup>	8.4 × 10 <sup>1</sup>
G-3	ts mutant	10 <sup>-4.9</sup>	1.2 × 10 <sup>3</sup>
G-9	ts <sup>+</sup> revertant	10 <sup>-0.2</sup>	2.4 × 10 <sup>1</sup>

<sup>a</sup> Random-bred golden Syrian hamsters (Lakeview Hamster Colony, Newfield, N.J.) were inoculated intracranially within 24 h of birth with 3 × 10<sup>9</sup> to 3 × 10<sup>4</sup> PFU in 0.05 ml. Virus infectivity was determined by assays at 33 C. Control animals were inoculated with an extract from uninfected BSC-1 cells. Each experimental group consisted of two to four litters. Litters were checked for mortalities 4 to 16 days postinoculation. Deaths prior to day 4 were attributed to needle trauma. The 50% lethal dose (LD<sub>50</sub>) was calculated by the Reed-Muench method based on the combined data from two independent experiments.

slightly more neurovirulent than CC can probably be attributed to the different passage histories of the G-9 and CC stocks.

The ability of CC, G-3, and G-9 to induce hydrocephalus in newborn hamster brains was tested by inoculating virus doses ranging from 3 to 30,000 PFU. As previously reported for ts G (6), G-3 induced hydrocephalus (Fig. 1B) at a high frequency in animals inoculated with large doses (54 and 84% for 3 × 10<sup>4</sup> and 3 × 10<sup>3</sup> PFU, respectively). The lower frequency of hydrocephalus observed at the highest dose was probably due to the lethality of this dose (73% mortality) and the inaccuracies of the inocula-

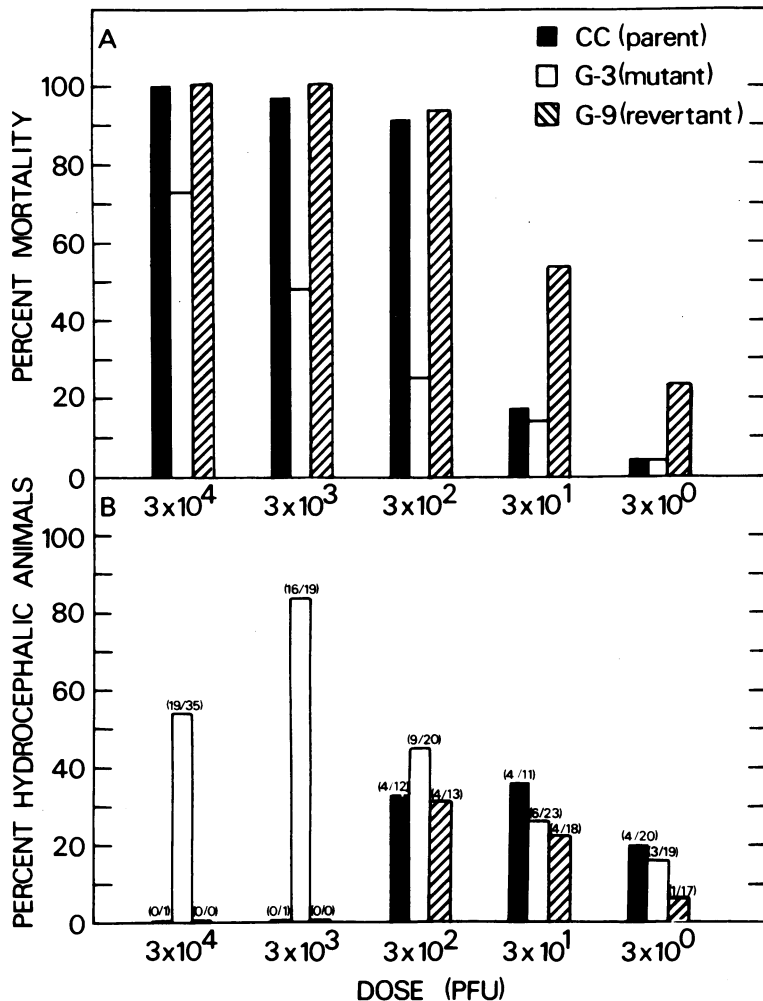


FIG. 1. Animals were inoculated and checked for mortalities as described in Table 1. For each dilution two to four litters (15 to 30 animals) were inoculated. From 12 to 16 days postinoculation any comatose animals were counted as mortalities, but were sacrificed before death. The brains were grossly examined for hydrocephalus as previously described (6). Animals with obvious dilation of the lateral ventricles were scored as positive for hydrocephalus. Such symptoms were not apparent earlier than 12 days postinoculation. Animals surviving beyond 16 days were sacrificed 18 to 60 days postinoculation, and the brains were examined. Results are based on the combined data from independent experiments for each dose. Fractions in parentheses indicate the number of hydrocephalic animals (numerator) and the total number of animals examined (denominator).

tion procedure. The incidence of hydrocephalus declined as the dosage was decreased further (Fig. 1B). Virus recovered by co-cultivation techniques (3) from four independent isolates of G-3-infected brains was temperature sensitive.

The ability of both CC and G-9 to cause hydrocephalus was also dependent on the dosage (Fig. 1B). With doses of  $3 \times 10^4$  or  $3 \times 10^3$  PFU, virtually all animals developed acute encephalitis and died within 10 days postinoculation (Fig. 1A). However, at lower doses a signifi-

cant proportion of the animals surviving 12 days or longer presented signs of hydrocephalus. The frequencies of hydrocephalus induced by low doses of CC, G-9, and G-3 were similar. The degree of hydrocephalus induced by the three strains ranged from slight to severe dilation of the lateral ventricles with disruption of normal brain structure. Virus recovered by co-cultivation from four independent isolates of G-9-infected brains was not temperature sensitive.

The results reported here more clearly define the neuropathogenic alteration caused by the ts G mutation. The co-reversion of temperature sensitivity and neurovirulence indicates that ts G probably contains a single mutational defect. Similar co-reversion was not observed for an attenuated strain of western equine encephalitis virus (9).

The results of previous studies suggested that the induction of hydrocephalus is a unique property of the ts G mutant (6). The present results demonstrate that this is not the case. Hydrocephalus evolves as a sequel to inoculation with appropriate doses of the revertant and parental strains and of a neurovirulent temperature-sensitive mutant, ts C (3), of measles virus (Breschkin and Rapp, unpublished observations). A similar dose-dependent induction of hydrocephalus was previously observed with a reovirus ts mutant but not with the parental strain (2).

A non-neurovirulent strain of mumps virus was previously reported to induce hydrocephalus (7, 8). In contrast, non-neurovirulent temperature-sensitive measles mutants do not cause hydrocephalus at a dose of  $3 \times 10^4$  PFU (1, 3). Thus, hydrocephalus probably does not develop as a sequel to inoculation of non-encephalitogenic doses of measles virus and appears to require some virus-induced insult to the brain. The tendency of ts G to cause hydrocephalus at a high frequency is probably a consequence of its mutational defect; because of this defect, high doses of ts G can induce a subacute encephalitis with resultant hydrocephalus. The nature of this defect is currently under investigation.

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