Evaluation of a Neonatal Rat Model for Prediction of Mumps Virus Neurovirulence in Humans

STEVEN A. RUBIN,1,* MIKHAIL PLETNIKOV,2 ROLF TAFFS,1 PHIL J. SNOY,1 DARWYN KOBASA,1 EARL G. BROWN,3 KATHRYN E. WRIGHT,3 AND KATHRYN M. CARBONE1,2,4

Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892; Departments of Psychiatry2 and Medicine,4 The Johns Hopkins University, Baltimore, Maryland 21218; and Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

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Neurovirulence of several mumps virus strains was assessed in a prototype rat neurovirulence test and compared to results obtained in the monkey neurovirulence test. The relative human neurovirulence of these strains was proportional to the severity of hydrocephalus in rats but not to lesion scores in the monkeys.

Neurovirulence testing is performed to demonstrate that attenuated mumps virus seeds used in the manufacture of vaccine lack the neurovirulence properties of wild-type mumps virus strains. Mumps virus neurovirulence testing as currently performed in monkeys has failed to discriminate between strains with known differences in human neurovirulence (2, 8, 27, 28). Similarly, tests in hamsters have also failed to reliably discriminate neurovirulent from nonneurovirulent human mumps virus strains (12, 19, 22, 34). The difficulty in evaluating the neurovirulence potential of mumps viruses may be reflected in reports of mumps virus central nervous system (CNS) infection causally linked to vaccination with some mumps virus vaccines (e.g., Urabe-AM 9, Leningrad-3, and Sofia-6) (3, 9, 10, 23, 24, 32). Thus, a validated mumps virus neurovirulence test with greater relevance to human disease remains an important public health objective.

Because neonatal rat brain is particularly sensitive to damage following perinatal virus infection (5, 6, 11, 13, 16, 25, 26, 31), it was hypothesized that neuropathology in rats neonatally inoculated with mumps virus may serve as a sensitive indicator of neurovirulence potential in the human CNS. Litters of 1-day-old Lewis rats were inoculated intracranially with 0.02 ml containing 10^2 PFU of the following mumps virus strains: (i) Jeryl Lynn vaccine (JL) (15); (ii) RIT 4385 vaccine (JL-RIT), cloned from JL by limiting dilution (33); (iii) Urabe-AM 9 vaccine (Ur-AM9) (35); (iv) Ur-1004, a cerebrospinal fluid isolate from a case of Ur-AM9 meningitis (7); (v) Kilham, a wild-type strain isolated from human breast milk and serially passaged in suckling hamster brain (20); (vi) Lo1, a wild-type strain isolated from the saliva of a patient with uncomplicated parotitis (1); and (vii) 88-1961, a wild-type strain isolated from the saliva of a patient with parotitis and symptoms of CNS infection.

Rats were euthanized on days 3, 6, 9, and 30 postinoculation, and brains were removed and either homogenized to determine viral titer by plaque assay (26) or fixed in 10% formalin for histological analysis. Two 3- to 4-mm-thick sagittal slices for histological analysis. Two 3- to 4-mm-thick sagittal slices were selected at a standard distance from either side of the anatomical midline from a fixed brain, paraffin embedded, sectioned, and stained with hematoxylin and eosin. The severity of hydrocephalus was determined as the percentage of the total brain cross-sectional area (excluding the cerebellum) occupied by the lateral ventricle on each of the two sections per rat using Image Pro Plus image analysis software (Media Cybernetics, Silver Spring, Md.). The mean percentage of hydrocephalus in each experimental group of rats was calculated and designated as the rat neurovirulence test (RNVT) score.

An example of the range in hydrocephalus severity is shown in Fig. 1, and the resultant RNVT scores are shown in Fig. 2A. Based on the RNVT scores, distinctions could be made in the relative neurovirulence (i) between vaccine and wild-type strains, (ii) among wild-type strains, and (iii) among vaccine strains. Differences in RNVT scores between vaccine and wild-type strains were significant (P < 0.001 for all scores compared) and paralleled the known clinical histories of these strains. While there have been no confirmed cases of strain JL- or JL-RIT-induced CNS infection, infection of the CNS occurs in up to 1% of Ur-AM9 vaccinees and in approximately 50% of cases of infection by wild-type mumps viruses (3, 4, 14). Among the wild-type strains, RNVT scores of strains 88-1961 and Kilham were greater than that of Lo1 (P ≤ 0.001 for all scores compared), consistent with the known neurovirulence of the three strains. Of note, the RNVT scores of strains 88-1961 and Kilham were equivalent, suggesting that while species adaptation may account for the high scores of the Kilham strain, neurovirulent human isolates can exhibit high RNVT scores in the absence of adaptation to rodents. Differences between Ur-AM9 and the JL-based vaccine strains were also significant (P < 0.02 for all scores compared) and consistent with their clinical histories as cited above. Differences in RNVT scores between JL and JL-RIT and between Ur-AM9 and Ur-1004 were not statistically significant.

A similar relationship between the virus strains and the resultant RNVT scores was observed at higher (10^4) and lower (10^3) doses of virus; however, there was a clear influence of virus dose on RNVT scores (data not shown). As to why the severity of hydrocephalus in rats is predictive of a strain's human neurovirulence potential is difficult to ascertain, since the pathogenesis of mumps virus-induced hydrocephalus is not well understood (29, 30). Studies in hamsters have associated mumps virus infection of ventricular ependymal cells with the development of hydrocephalus (17, 18), suggesting that hydrocephalus severity may reflect the ability of the viral strains to replicate in rat brain. Indeed, as shown in Fig. 2B, a direct...
correlation between viral titers and RNVT scores for different strains of mumps virus was demonstrated.

Recent reports have indicated that the monkey neurovirulence test (MNVT) is not sufficiently predictive of mumps virus neurovirulence in humans (2, 27). However, based on the observed influence of virus dose on RNVT scores, it was conceivable that the use of higher or lower doses of mumps virus might yield responses in monkeys better correlating with neurovirulence in humans. Consequently, Macaca mulatta rhesus monkeys were inoculated intrathalamically with 1.0 ml (0.5 ml per thalamus) containing $10^{3.5}$, $10^{4.5}$, and $10^{5.5}$ PFU per ml of strain JL, Lo1, or 88-1961. These doses closely approximated those used in the rat study on a per gram of brain tissue basis. Monkeys were euthanized on day 17 postinoculation, and brains were removed, fixed in 10% formalin, blocked, embedded in paraffin, sectioned, and stained with gallocyanin as previously described (21). MNVT scores were determined based on mumps virus-specific inflammation and neuronal destruction as previously described (27). Of note, monkeys do not develop measurable hydrocephalus as a consequence of mumps virus infection.

At a dose of $10^{3.5}$, all mumps virus strains resulted in similar MNVT scores (Fig. 3). At higher virus doses, MNVT scores for strain 88-1961 were greater than both Lo1 and JL MNVT scores, with the differences being statistically significant in the

FIG. 1. Representative sagittal brain sections from rats inoculated with mumps virus. Arrows indicate the lateral ventricle. (A) No hydrocephalus; (B) mildly enlarged ventricle occupying 6% of the total brain cross-sectional area; (C) moderately enlarged ventricle occupying 12% of the total brain cross-sectional area; and (D) severely enlarged ventricle occupying 26% of the total brain cross-sectional area. The sections were stained with hematoxylin and eosin. Magnification, ×1.5.

FIG. 2. Mumps virus strain-specific hydrocephalus and viral burden. (A) The RNVT scores track with the known clinical history of all strains. Each bar represents measurements obtained from 12 to 18 rats. (B) Titer of infectious virus per gram of rat brain on days 0, 3, 6, and 9 postinoculation with strain JL (filled circles), Ur-AM9 (open circles), Lo1 (filled triangles), and 88-1961 (open triangles).

FIG. 3. MNVT scores in monkeys inoculated with $10^{3.5}$, $10^{4.5}$, and $10^{5.5}$ PFU of strain JL (filled circles), Lo1 (open circles), and 88-1961 (filled triangles). Each data point represents measurements obtained from five monkeys.
former comparison ($P \leq 0.02$ for all scores compared) but not in the latter comparison ($P > 0.08$ for all scores compared). Thus, at $10^{2.5}$ and $10^{3.5}$ PFU of virus, there was a trend for the MNVT to discriminate between highly neurovirulent (88-1961) and less neurovirulent (Lo1 and JL) strains. However, at all doses tested, there was also a trend of increased neurovirulence of JL relative to Lo1, indicating an overall inability of the MNVT to reliably assess human neurovirulence. Therefore, pending verification of results in multicenter collaborative investigations, the data presented here support the replacement of the MNVT with the RNVT.

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