The Jeryl Lynn vaccine strain of mumps virus is a mixture of two distinct isolates


National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, U.K.

Sequence analysis of the region of the mumps virus genome encoding the putative small hydrophobic protein gene confirms that it is a highly variable region. Jeryl Lynn, the mumps vaccine strain used in the U.K., is shown to be a mixture of two closely related viruses, both probably of American origin.

Mumps virus is a member of the Paramyxoviridae family of the paramyxovirus genus. It consists of a single strand of negative-sense genomic RNA of about 15.3 kb in a lipid-containing virion composed of two surface glycoproteins, the haemagglutinin-neuraminidase (HN) and the fusion (F) proteins, and four core proteins, the nucleoprotein (N), phosphoprotein (P), matrix protein (M) and large protein (L). The virus is responsible for mumps, a common childhood disease, although about one third of infections are believed to be asymptomatic.

Parotitis is the most common symptom in the remaining cases but infection of the central nervous system is common. In the United Kingdom before the widespread use of vaccines, mumps was responsible for 1500 to 2000 cases of meningitis and encephalitis requiring hospital treatment per year.

Mumps vaccine is given to children together with measles and rubella vaccines at or after 12 months of age. Two strains of live attenuated mumps virus have been used in vaccines in the U.K., namely the Urabe strain, derived from a wild-type Japanese isolate by passage in egg amnion (Yamanishi, 1970) and the Jeryl Lynn strain, derived by tissue culture passage of a wild-type American isolate (Buynak & Hilleman, 1966). Vaccines other than those based on live attenuated virus strains are not available for mumps.

The Urabe strain has been implicated in cases of aseptic meningitis following vaccination at estimated frequencies ranging from about one in 100000 (Hockin & Furesz, 1988) to one in 3800 (Colville & Pugh, 1992) and purchase of vaccines containing this strain was suspended in the U.K. although the cases resolve within a few days with no known sequelae. In contrast, the Jeryl Lynn strain has not been shown convincingly to be involved in post-vaccination meningitis (Nalin, 1989, 1992). Isolates from cerebrospinal fluid of children hospitalized with meningitis have been shown to be derived from the Urabe strain by sequence analysis of the F gene (Forsey et al., 1990), the P gene (Yamada et al., 1990) and the HN gene (Brown et al., 1991). We have previously reported that the region of the genome encoding the putative small hydrophobic protein (SH) is highly variable between isolates (Turner et al., 1991). In this paper we confirm and extend these observations to a larger number of isolates, but also report that Jeryl Lynn vaccines as used in the U.K. are a mixture of two distinct but related viruses.

Viruses were grown and plaque-purified where appropriate on Vero cells using isolates available at NIBSC. Virus was concentrated with 4% polyethylene glycol 600/0.5 M-NaCl, RNA was extracted as described (Turner et al., 1991), and transcribed into cDNA using reverse transcriptase and primer P1 (5' TCAGAAA-GGATCCACTTCAAAAT 3'). Primer P1 corresponds to nucleotides 6088 to 6113 of the genome. Sequences were then amplified by PCR (Turner et al., 1991) using primer P1 and P2 (5' ATAACTGAAT-TCATTACTCCACAGC 3') corresponding to nucleotides 6865 to 6890. The sequences of P1 and P2 were slightly modified from that of the mumps virus genome to introduce EcoRI and BamHI restriction sites for subsequent cloning into M13 mpl8/mpl9 for sequencing. In some cases the PCR fragment was sequenced directly using T7 polymerase (Turner et al., 1991).

The similarity between sequences was illustrated by construction of a phenogram, or tree, using an agglomerative distance matrix approach (Nei, 1987).
branches of the tree are not intended to imply an evolutionary path. The tree simply illustrates similarity between sequences.

Vero cells were infected with the Jeryl Lynn vaccine strain of mumps virus, and the harvest was amplified by PCR over a region of the genome including the SH gene and part of the HN gene. Ambiguities were observed at a number of positions when the product was sequenced (P. C. Turner et al., unpublished observation) which were not observed in the sequence of the Urabe strain (Turner et al., 1991).

Plaques were picked on Vero cells from the unpassaged bulk vaccine from the manufacturer and pools prepared in Vero cells. Amplification and sequencing of the product cloned into M13 revealed that pools grown from separate plaques were of two sequences (designated JL2 and JL5) differing at a number of positions, as shown in Fig. 1. The 618 nucleotide sequence covered the putative SH gene, which has not yet been shown to be expressed, and a part of the HN gene. Of the 20 differences between the two strains one was in the 5' non-coding region of the SH gene, nine in the coding region and four in the 3' non-coding region, representing 0%, 2.3% and 4.4% and 2%, 5.3% and 4.1% divergence respectively from the published SBL-1 SH gene sequence (Elliott et al., 1989). Four differences were located in the 5' non-coding region of the HN gene, and two in the 222 nucleotides of the coding region sequenced, representing 3.7% and 0.9% divergence between the two variants respectively. The changes resulted in four predicted amino acid differences in the coding region of the SH gene, and no coding change in the HN gene. Similarly the M gene sequence of both JL-2 and JL-5 variants shows very little divergence (M. A. Afzal et al., unpublished results).

Comparison of the sequences of JL2 and JL5 with the sequence of the Urabe genome over the same region revealed one and two nucleotide differences respectively in the 5' non-coding region of the SH gene, representing 2% and 4% divergence, 25 and 23 differences in the
coding region, representing 14.6% and 13.5% divergence, and 16 and 17 differences in the 3' non-coding region, representing 16.5% and 17.5% divergence. The differences in the coding region would give rise to 13 and 11 amino acid differences from the Urabe sequence if the SH protein is translated. In the HN gene, comparison of JL2 and JL5 with Urabe showed the presence of 19 and 17 nucleotide differences in the 5' non-coding region representing 24% and 21% divergence. The 20 nucleotide differences in the portion of the coding region of the HN gene sequenced (9% divergence) for both variants are predicted to give rise to six amino acid changes (8.1% divergence). The base at residue 92 in Urabe was found to be a guanosine and not an adenosine as previously reported (Turner et al., 1991). The conservation of the 5' non-coding region of the SH gene compared to the coding and 3' non-coding region of the SH gene and the 5' non-coding region of the HN gene is consistent with previous findings (Turner et al., 1991).

The sequence designated JL2 corresponds to that published for Jeryl Lynn by others (Takeuchi et al., 1991). A number of M13 clones were derived from pools grown in Vero cells from five independent plaques picked from the vaccine bulk. The sequence designated JL2 corresponded to virus from all three M13 clones obtained from one plaque-purified pool whereas that designated JL5 was observed in virus from the 17 M13 clones obtained from the other four.

The plaque-purified pools were the product of two additional Vero passages of the vaccine bulk which might affect the ratio observed. A pool of virus was grown on Vero cells from the vaccine bulk and genomic RNA was amplified, cloned into M13 and sequenced. Of 24 clones sequenced, four were of the JL2 and 20 of the JL5 sequence, giving a ratio of 1:5, comparable to that seen with the plaque-derived isolates.

Finally, unpassaged virus from four vaccine bulks supplied by the manufacturer were examined directly by the same method. None of two, one of five, one of two and one of eight clones had the sequence of JL2, whereas the remainder had the sequence of JL5. Overall of 18 clones, three had the sequence of JL2 giving a ratio of JL2 to JL5 of 1:5. The ratio of JL2 to JL5 sequences was therefore very similar in all preparations examined, and the two sequences could be readily identified in three of four unpassaged vaccine bulks. This suggested that passage in Vero cells was not selecting strongly for one of the two virus strains. However, it is possible that some host cell lines might select one of the variants; Takeuchi et al. (1991) have reported on Jeryl Lynn vaccine virus further propagated in chick embryo fibroblasts and in HeLa cells and do not mention the existence of the JL5-like variant in virus preparations.

Sequences of the SH gene were determined for a number of other isolates, including several from the U.K. and several shown to be related to the Urabe vaccine by sequencing the F gene. The sequences determined at NIBSC and published sequences were compared to give the tree shown in Fig. 2, which illustrates that JL2 and JL5 form a cluster with the Kilham, SBL and Enders strains. Two other clusters were identified, one composed of isolates from the U.K. and the RW isolate (American), and the other of Japanese isolates and Urabe-derived strains. The relationships identified in Fig. 2 were in general consistent with those identified elsewhere based on comparison of the F gene sequence (Forsey et al., 1990). The exception was the Enders strain which clustered with British isolates according to the sequence of its F gene (Elliott, 1988) but with Jeryl Lynn and SBL on the basis of the sequence of its SH gene. Greater variation was seen in the SH gene than in the F gene. In particular, JL2 and JL5 differed in the sequence of their SH gene but not within the region of the F gene examined. All Urabe-derived isolates so far examined were of the same sequence in the F gene, whereas some differed from Urabe and from each other by one nucleotide in the SH gene. By analogy with poliovirus (Rico-Hesse et al., 1987), viruses with a common
geographical origin might be expected to form a cluster, and Enders and the two Jeryl Lynn strains group together, consistent with their American origin. The SBL strain originated from Sweden, however, and also forms part of the cluster; RW, an American strain, clusters with the British isolates. The reasons for these relationships are not clear, but the two Jeryl Lynn strains are very similar.

Based on the relationships illustrated in Fig. 2, it is unlikely that the mixed population found in the Jeryl Lynn strain is the result of laboratory contamination at NIBSC, as no other virus having the sequence of JL2 or JL5 has been identified, and the cluster suggests that both JL2 and JL5 originate from a common epidemiological region, presumably America. It is conceivable that the divergence between the strains could have originated in the course of passaging a common isolate, but in view of the large number of differences between the sequences it seems more likely that they derive from two separate wild-type strains.

The clinical significance, if any, of a heterogeneity of this magnitude in a vaccine strain is under investigation. Preliminary data suggest that the Jeryl Lynn strain provokes a more broadly reactive serological response in vaccinees than does the Urabe strain (T. Forsey, unpublished). It is also possible that the two strains differ in their virulence for recipients, in which case the ratio of the two viruses may be of significance to the safety of the vaccine.

Jeryl Lynn vaccine is manufactured under strictly controlled passage methodology to ensure a consistently reproducible immunogenicity, safety and tolerability profile. Virological neutralization tests are included among the release specification to ensure uniform product performance. This product profile can be continued into the foreseeable future by maintaining the rigorous preparation steps and constant technology by which the filled vaccine is prepared from the master seed and the filled vials are released. Although RNA viruses are well known to develop variant subpopulations, the presence of a constant ratio of two strains in this vaccine is surprising. However, there is every reason to believe that the product will remain consistent in quality and its excellent record of clinical safety and efficacy will be maintained.

We thank Elizabeth Trainor for typing the manuscript.

References


(Received 21 October 1992; Accepted 1 December 1992)