Differentiation of vaccine and wild mumps viruses using the polymerase chain reaction and dideoxynucleotide sequencing

Timothy Forsey,* Jane A. Mawn, Phillip J. Yates, Maureen L. Bentley and Philip D. Minor

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

Parts of the F gene from 16 mumps viruses derived from vaccines and clinical isolates were amplified using the polymerase chain reaction and their nucleotide sequences were determined. Over a region of 111 nucleotides, eight regions of variability were detected with a maximum of six (5.4%) changes occurring between any two virus strains. The Jeryl Lynn and Urabe vaccine strains were clearly different from each other and from wild virus isolated from cases of non-vaccine-associated mumps. In contrast, viruses isolated from the cerebrospinal fluid and throat in cases of meningitis and parotitis following vaccination with the Urabe strain were identical to this strain. We conclude that the vaccine was the source of these infections.

Routine immunization of children with mumps vaccine was introduced into the U.K. in October 1988 in the form of a measles–mumps–rubella (MMR) triple vaccine. At present the MMR vaccines used include either the Jeryl Lynn strain (Buynak & Hilleman, 1966) or the Urabe strain (Yamanishi et al., 1970) of mumps virus. It has been claimed that the incidence of central nervous system reactions to the Jeryl Lynn strain is far less than that to the Urabe strain (Nalin, 1989). However, in a small clinical trial comparing these two vaccines in Finland no significant difference in adverse reactions was noted (Vesikari et al., 1984). Since the introduction of MMR in the U.K., three cases of mumps meningitis, confirmed by isolating virus from cerebrospinal fluid (CSF), have been reported in children following vaccination with the Urabe strain (Gray & Burns, 1989; Murray & Lewis, 1989). This represents an incidence of adverse reactions in the U.K. of approximately one per 800000 doses distributed. Eight similar cases have been reported in Canada (Hockin & Furesz, 1988) and one in West Germany (von Muhlendahl, 1989). It has been estimated that if all suspected vaccine-associated cases of mumps meningitis, confirmed by isolating virus from cerebrospinal fluid (CSF), have been reported in children following vaccination with the Urabe strain (Gray & Burns, 1989; Murray & Lewis, 1989). This represents an incidence of adverse reactions in the U.K. of approximately one per 800000 doses distributed. Eight similar cases have been reported in Canada (Hockin & Furesz, 1988) and one in West Germany (von Muhlendahl, 1989). It has been estimated that if all suspected vaccine-associated cases of mumps meningitis were in fact due to the vaccine then the incidence of mumps meningitis would be approximately one per 100000 doses. In the U.K. this would represent fewer than 10 cases per year. In contrast, infection by wild mumps virus is estimated to be responsible for between 1500 and 2000 hospital admissions per year. This figure includes all complications of mumps, such as meningitis, orchitis, etc. It is important to establish whether post-vaccination cases were due to the vaccine virus or were caused by coincidental exposure to wild mumps virus circulating at that time. Monoclonal antibody studies have demonstrated some antigenic differences between mumps virus strains (Server et al., 1982; Orvell, 1984; Rydbeck et al., 1986) but no clear marker exists for differentiating vaccine virus from wild virus. We have attempted to find such markers by sequencing part of the F gene from vaccine strains and clinical isolates of the virus. Recent reports have shown that sequence differences between strains do occur in this region (Elliott et al., 1989) and also in the P gene (Yamada et al., 1989).

Mumps virus isolates were obtained from non-vaccine-associated cases of parotitis and meningitis and from similar cases which followed vaccination with the Urabe strain (Table 1). Isolates were obtained as tissue culture fluid from low passage of virus in Vero cells. Vaccine strains were similarly passaged in this cell line. Total RNA was extracted from tissue culture fluid (Macadam et al., 1989) and cDNA transcripts were made from viral RNA. These were amplified by the polymerase chain reaction (PCR) following a protocol adapted from that supplied with the Gene Amp Kit (Perkin-Elmer Cetus). Oligonucleotide primers corresponding to regions found on the published sequence for the F gene of the RW strain (Waxham et al., 1987) were synthesized on an Applied Biosystems DNA synthesizer. Primer A46 (5' TCGCCCTCACTG 3', mRNA sense; nucleotide positions 397 to 411) was used to synthesize cDNA. Primer pair A54 (5' ATCTTAGAGATCGGG 3', mRNA sense; positions 1108 to 1122) and B18 (5' GCTCAAAACTAAGGC 3', vRNA sense;
Table 1. Source of mumps virus isolates

<table>
<thead>
<tr>
<th>Designation</th>
<th>Site of Clinical condition</th>
<th>isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mu/Bristol1/1989</td>
<td>(BS1) Parotitis</td>
<td>Throat</td>
</tr>
<tr>
<td>Mu/Edinburgh4/1988</td>
<td>(ED4) Meningitis</td>
<td>CSF</td>
</tr>
<tr>
<td>Mu/Edinburgh6/1988</td>
<td>(ED6) Sore throat</td>
<td>Nose/throat</td>
</tr>
<tr>
<td>Mu/Edinburgh7/1989</td>
<td>(ED7) Post-vaccination meningitis</td>
<td>CSF</td>
</tr>
<tr>
<td>Mu/London1/1989</td>
<td>(LO1) Parotitis</td>
<td>Saliva</td>
</tr>
<tr>
<td>Mu/Nottingham1/1989</td>
<td>(NT1) Post-vaccination meningitis</td>
<td>CSF</td>
</tr>
<tr>
<td>Mu/Canada1/1987</td>
<td>(CA1) Post-vaccination parotitis</td>
<td>Throat</td>
</tr>
<tr>
<td>Mu/Canada2/1987</td>
<td>(CA2) Post-vaccination parotitis</td>
<td>Throat</td>
</tr>
<tr>
<td>Mu/Canada3/1987</td>
<td>(CA3) Post-vaccination parotitis</td>
<td>Throat</td>
</tr>
<tr>
<td>Mu/Canada4/1987</td>
<td>(CA4) Post-vaccination meningitis</td>
<td>CSF</td>
</tr>
<tr>
<td>Mu/Canada5/1987</td>
<td>(CA5) Post-vaccination meningitis</td>
<td>CSF</td>
</tr>
<tr>
<td>Mu/Canada6/1987</td>
<td>(CA6) Post-vaccination meningitis</td>
<td>CSF</td>
</tr>
<tr>
<td>Mu/Canada7/1988</td>
<td>(CA7) Parotitis</td>
<td>Throat</td>
</tr>
<tr>
<td>Mu/Canada8/1988</td>
<td>(CA8) Parotitis</td>
<td>Throat</td>
</tr>
</tbody>
</table>

positions 1591 to 1605) and primer pair C48 (5' GTAGCACTGGATGGA 3', mRNA sense; positions 1231 to 1245) and C196 (5' ACTCACAGATTGGAG 3', vRNA sense; positions 1543 to 1557) were used in PCR.

dsDNA products of PCR were visualized on agarose gels stained with ethidium bromide. Their specificity was checked by Southern blotting using an internal oligonucleotide as probe. They were purified on agarose gels (Dretzen et al., 1981) and sequenced using primer A54 or C48 in a dideoxynucleotide chain termination reaction (Sanger et al., 1987) incorporating dimethyl sulphoxide to prevent strand re-annealing (Winship, 1989).

Initially a region of 102 bases from several virus strains was sequenced using primer A54. In this region, only two nucleotide changes were seen between the Urabe and Jeryl Lynn vaccine strains and only one change between Urabe and the U.K. wild-type viruses (data not shown). In contrast, using primer C48 a region of 111 bases was sequenced which showed eight regions of change between the various mumps strains. The results of sequencing 16 isolates with this primer are compared to the published sequences of four other mumps viruses in Table 2. Genomic RNA from several strains was reverse-transcribed, amplified and sequenced on more than one occasion and gave reproducible results. In addition, the sequence of the Jeryl Lynn strain obtained after PCR was identical to that obtained from virus grown in bulk in eggs and sequenced directly. The four clinical isolates from cases of non-vaccine-associated mumps in the U.K. were identical over the region sequenced, suggesting that closely related strains of wild virus were circulating over a wide area of the U.K. between 1988 and 1989. All these strains were similar to one wild-type virus from Canada and differed by a single nucleotide from the other Canadian wild virus. These strains differed from the Jeryl Lynn and Urabe vaccine strains and from the published sequences of RW, SBL and Miyahara (Waxham et al., 1987; Elliott et al., 1989; Takeuchi et al., 1989). However, these wild strains were similar to the published sequence of a tissue culture-adapted Enders strain (Elliott, 1988) which was originally isolated in 1945 from a case of parotitis in the U.S.A. (Enders et al., 1946). This strain has never been used in our laboratory.

The eight isolates from vaccine-associated cases of meningitis and parotitis, two in the U.K. and six in Canada (Table 1) were clearly different from the U.K. and Canadian wild-type viruses, from SBL and from the vaccine strains Jeryl Lynn and Miyahara. However, they were identical to the Urabe vaccine strain indicating that this vaccine was the source of these isolates.

Of the eight regions of nucleotide change seen in these strains, only those at positions 1289 and 1348 produced coding changes. At the first position Urabe and Urabe vaccine-associated isolates possess an asparagine residue whereas all other strains possess a serine. At the second position, RW and Jeryl Lynn possess threonine residues, SBL a serine and the other strains an alanine residue.

In a comparison of sequence divergence between pairs of strains we found a maximum of six (5-4%) nucleotide changes occurring. The relatedness of strains when Miyahara is taken as the consensus sequence is shown in Fig. 1. Four groups were apparent: Jeryl Lynn and SBL; Miyahara, Urabe and Urabe-associated isolates; Enders and wild-type isolates; RW.

A similarity in the antigenic characteristics of Jeryl Lynn and SBL has previously been found using monoclonal antibodies (Rydebeck et al., 1986). It is not surprising that Miyahara and Urabe are similar since both are vaccine strains from Japan. However, we have no explanation for the similarity of U.K. and Canadian
null
seven (3.8%) changes between Enders and Jeryl Lynn over 183 nucleotides of the P gene whereas we found six (5.4%) changes between these two strains over 111 nucleotides of the F gene. From these results it appears that a similar degree of variability (4 or 5%) exists in both the F and P genes of these mumps viruses.

Sequence data in this report were derived from a small region of the F gene of mumps viruses. Studies are presently being extended to cover other areas of the viral genome.

References


(Received 26 October 1989; Accepted 13 December 1989)