Short communication

Efficient isolation of mumps virus from a community outbreak using the marmoset lymphoblastoid cell line B95a

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Abstract

The incidence of mumps infection in the UK was reduced greatly by vaccination as a component of MMR vaccine, but cases and outbreaks continue to occur. Although in specialised laboratories RT-PCR is available for detection of mumps virus RNA in clinical samples, in routine laboratories virus isolation remains a standard method for diagnosing mumps virus infection. Furthermore, isolation of mumps virus strains circulating currently is important for monitoring the ability of vaccine-induced antibody to neutralise any genotypes recognised recently and to detect any changes in phenotype. In this study we compared rhesus monkey kidney (RMK) cells with the cell line B95a for mumps virus isolation from twenty throat swabs collected during a mumps outbreak in a religious community with low MMR coverage. Mumps virus was isolated from eight cases (40%), six were positive in both cell cultures and two in only one, all positive samples being collected within 2 days of onset. Virus growth in B95a cells was detected by the production of a syncytial cytopathic effect, and confirmed by an indirect fluorescent antibody test using a mumps monoclonal antibody. The B95a cell line was found to be equally as sensitive for mumps isolation as RMK cells, which are regarded as the ‘gold standard’, thus providing an alternative to the use of primary animal cell culture.

Keywords: Mumps virus isolation; B98a cells; RMK cells; Cytopathic effect; Immunofluorescence

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The incidence of mumps, once a common childhood infection in the UK, was greatly reduced by the introduction of mumps vaccination as a component of MMR vaccine in 1988 (Gay et al., 1997). However, due to suboptimal vaccine coverage and primary and secondary vaccine failure, mumps continues to circulate and cause outbreaks particularly in adolescents (Anonymous, 2000). Molecular epidemiological studies have shown that several different genotypes of mumps virus are now circulating in the UK, and that the index case in several of the outbreaks had become in-
ected outside the UK with a genotype circulating elsewhere (Jin et al., 1999).

Although in specialised laboratories techniques are now available for the diagnosis of mumps infection by detection of IgM antibody or RNA in saliva, the standard methods of serum antibody detection and virus isolation from throat swab or urine are still commonly used. Primary monkey kidney cell cultures are considered the optimal cells in which to isolate mumps virus, but continuous mammalian cell lines, e.g. Vero, are also used (Wolinsky, 1996).

We describe here the isolation of mumps virus from cases occurring during a community outbreak, using both secondary rhesus monkey kidney cells (RMK; obtained as a primary suspension from BioWhittaker UK, Wokingham, UK) and the EBV-transformed marmoset lymphoblastoid cell line B95a (Kobune et al., 1990; obtained from the European Collection of Cell Cultures, Centre for Applied Microbiology and Research, Salisbury, UK; accession no. 99093030). B98a cells, derived from the suspension culture B958, grow as a monolayer and are highly susceptible to measles virus and provide currently the cell cultures of choice for measles virus isolation.

An outbreak of mumps occurred in 1998 and 1999 in an orthodox religious community in North London in which the MMR vaccine coverage was low (van den Bosch et al., 2000). One hundred and forty-four cases were notified in patients aged between 9 months and 39 years, and the diagnosis was confirmed virologically by salivary assays in 51 (82%) of 62 tested. Throat swabs were collected in virus transport medium from 23 cases between 1 and 14 days after onset of parotitis, transported to the laboratory and frozen at −70°C until tested by RT-PCR for mumps virus RNA and virus isolation. Twenty samples were positive by RT-PCR and the virus typed as genotype C (van den Bosch et al., 2000).

Throat swab samples were thawed and 100 μl inoculated into tube cultures of both secondary RMK and B95a cells. All tubes were rolled at 33°C and inspected regularly for cytopathic effect (cpe). The inoculated RMK cells were also tested for haemadsorption using 0.4% guinea pig erythrocytes. The culture fluid was replaced with fresh maintenance medium every 3–4 days for B95a cells (Dulbecco’s modified Eagle’s medium with sodium bicarbonate, supplemented with 2% foetal bovine serum and 50 μg/ml gentamicin) or weekly for RMK cells (Eagle’s minimal essential medium with Earle’s salts and HEPES, supplemented with non-essential amino acids and 50 μg/ml gentamicin). If virus growth was not detected after 14–16 days, the cells were scraped into the medium, a blind passage carried out, and incubation continued for a further 2 weeks.

Mumps virus was isolated from eight of the 20 RT-PCR positive throat swabs tested; six samples were positive in both types of cell culture and two in only one type (Table 1). A high proportion of swabs (eight of 11; 73%) taken within 2 days of onset yielded infectious virus, but all those collected later were negative. The cpe usually began in both types of culture between 4 and 7 days after inoculation. However, in the sample in which only the RMK cells were positive, haemadsorption was demonstrated at 10 days followed by a cpe by 16 days, and in the sample only positive in B98a cells the cpe was delayed until day 11.

Table 1
Isolation of mumps virus from throat swabs in B95a and RMK cell cultures

<table>
<thead>
<tr>
<th>Days after onset of symptoms</th>
<th>B95a</th>
<th>+</th>
<th>+</th>
<th>–</th>
<th>–</th>
<th>No. specimens tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3–7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>8–14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
The growth of virus in RMK cells was detected by the appearance of typical syncytia, and confirmed by haemadsorption with guinea pig erythrocytes; electron microscopy of the culture fluid (courtesy of Dr Hazel Appleton) showed characteristic paramyxovirus particles and pieces of nucleoprotein helix. The cpe in B95a cells consisted of smaller, granular oval syncytia and also very elongated syncytial areas (Fig. 1a). Mumps virus was confirmed in these cells by an indirect immunofluorescent antibody test. Infected cultures were trypsinised, the cells washed in phosphate-buffered saline (PBS), and spotted onto multiwell slides. After fixing in cold acetone for 10 min the cells were stained with a monoclonal antibody to mumps virus nucleocapsid (1668, kindly provided by Dr Morag Ferguson, National Institute for Biological Standards, UK) followed by FITC-conjugated rabbit anti-mouse immunoglobulin and examined by fluorescence microscopy (Fig. 2a).

The effects of mumps genomic variation on the antigenic or phenotypic properties, e.g. virulence, of the virus are unknown. It is, therefore, important to obtain viral isolates to study these properties and to monitor the ability of vaccine-induced antibody to neutralise any newly recognised genotypes. We have shown that the B95a cell line, which is currently used for measles virus isolation, is as sensitive as secondary RMK for primary isolation of mumps virus, at least for the genotype C mumps virus strain in the outbreak we studied. Furthermore, the use of a cell line overcomes...
ethical problems associated with the use of primary animal cell cultures.

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References