A measles virus isolate from a child with Kawasaki disease: sequence comparison with contemporaneous isolates from 'classical' cases

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We examined the relationship between a measles virus isolate from a child with Kawasaki disease and two contemporaneous wild-type isolates from children with 'classical' measles and the Schwarz vaccine strain. Sequence analysis of 3118 bp from the nucleoprotein, matrix, fusion and haemagglutinin genes of each virus revealed that the isolate from the child with Kawasaki disease was not related to measles vaccine strains and did not contain any of the marked abnormalities previously found in subacute sclerosing panencephalitis isolates, but was more akin to wild-type isolates currently circulating in the U.K. A comparison of our sequences with those obtained from earlier wild-type U.K. isolates suggests significant evolution of measles virus in the U.K. over the last decade.

Mucocutaneous lymph node syndrome (Kawasaki disease; Kawasaki et al., 1974) is a systemic necrotizing vasculitis occurring mainly in young children (Rowley et al., 1988; Tizard et al., 1991). Although epidemiological studies point to an infectious aetiology, no single microorganism has so far been isolated consistently (see Melish & Marchette, 1991 for a review), and a proposed retrovirus aetiology (Burns et al., 1986; Shulman & Rowley, 1986) has been disputed (Melish et al., 1988). We have recently isolated a measles virus from a child with Kawasaki disease (Whitby et al., 1991) and, similarly, an earlier report mentioned a measles virus isolated from a child suffering from Kawasaki disease a few weeks after measles vaccination (Melish et al., 1988). Therefore we sequenced parts of the genome of our isolate and compared the sequence to those of measles virus isolates obtained at about the same time from children with 'classical' measles, as well as to those of previously sequenced isolates to establish its relationship to circulating 'wild-type' viruses or vaccine-related strains.

As reported in detail elsewhere (Whitby et al., 1991), isolate TT was from a 9-month-old child fulfilling all the criteria necessary for the diagnosis of Kawasaki disease, including coronary artery dilatations. This child was resident in London, U.K. and of Caribbean descent. Anti-measles virus IgG and IgM antibodies were not detected in a first serum sample, but serum was IgG-positive 4 months later, indicating seroconversion (Whitby et al., 1991). For virus isolation, peripheral blood mononuclear cells (PBMCs) were cultured in RPMI, 10% foetal calf serum, 1 μg/ml phytohaemagglutinin and 10 units (U)/ml interleukin 2, and subsequently cocultivated with the Raji cell line, in which the isolate induced syncytium formation. For comparison of this isolate with contemporaneous 'classical' measles viruses, two isolates (CL and SE) from a small outbreak of measles in the West Midlands, U.K. were made by the same method. Blood samples from both these children (6 and 5 years old, respectively) were taken within 20 days of each other, in April and May 1988. All three isolates also grew and induced syncytia in adherent cell lines, e.g. HeLa.

We sequenced a total of 3118 bp from each virus isolate, as well as from the Schwarz vaccine strain, representing approximately 20% of the total genome, by amplifying cDNA fragments using the polymerase chain reaction (PCR) followed by direct sequencing. cDNA was synthesized from RNA extracted from infected Raji cells by adding 100 ng of an antisense primer (Table 1) to 3 to 5 μg of total RNA in 10 μl of 10 mM-HEPES pH 6.9, 0.2 mM-EDTA. This mixture was overlaid with 30 μl of paraffin oil, heated to 90 °C for 2 min and chilled on ice for 5 min. To this was added 4 μl 5 x RT buffer (250 mM-Tris–HCl pH 7.5, 375 mM-KCl, 15 mM MgCl₂, 50 mM-DTT), 1 μl 40 mM-dNTP, 20 U Moloney murine leukaemia virus reverse transcriptase and 5 μl H₂O, and the reaction mixture was incubated at 37 °C for 90 min.
Table 1. **Primers used for cDNA synthesis, PCR amplification and sequence analysis**

<table>
<thead>
<tr>
<th>Region analysed (length, position in measles virus genome)</th>
<th>Primer</th>
<th>Sequence (position in measles virus genome)</th>
<th>Coding/non-coding strand (+/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP gene (291 bp, 1369 to 1660)</td>
<td>MN1</td>
<td>AAGCCCAAGTATCATTTTCT (1349 to 1368)</td>
<td>+</td>
</tr>
<tr>
<td>M gene (905 bp, 3492 to 4397)</td>
<td>MM1</td>
<td>CTTTATGTACATGTTTCTGCT (3584 to 3604)</td>
<td>+</td>
</tr>
<tr>
<td>H gene (998 bp, 7837 to 8835)</td>
<td>MH1</td>
<td>TGGGACACCAAGAACAACCAAT (7784 to 7803)</td>
<td>+</td>
</tr>
<tr>
<td>F gene (890 bp, 6259 to 7149)</td>
<td>MF2</td>
<td>ATACAGTGGAGGTATTACCT (6206 to 6225)</td>
<td>+</td>
</tr>
</tbody>
</table>

cDNA (5 μl) was then amplified by PCR as described previously (Saiki et al., 1988) using 200 ng of a suitable primer pair (see below), in a total volume of 50 μl, and 30 cycles of amplification (96 °C, 1 min; 45 °C, 1 min; 72 °C, 1 min). Reaction products were separated on an agarose gel, purified using Geneclean and sequenced directly using the primers listed in Table 1 and a method described previously (Winship et al., 1989). In the case of the nucleoprotein (NP) gene, appropriate amplification products were cloned into the SmaI site of the pBluescript plasmid and a minimum of three clones was sequenced on both strands. The 291 bp fragment of the NP gene (nucleotides 1369 to 1660; amplified and sequenced with primers MN1 and MN2; Table 1) encodes a region in the C-terminal part of NP which has previously been shown to be divergent in different morbilliviruses (Rozenblatt et al., 1985) and has been used more recently to demonstrate the existence of several distinct lineages of measles virus (Taylor et al., 1991). The region of the matrix (M) gene (nucleotides 87 to 992), analysed with primers MM1 and MM3, MM2 and MM3, and MM5 and MM4, corresponds to most of the coding region of this gene with the exception of the first 54 and the last 48 nucleotides, and was chosen because of its variability in isolates from subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis cases (Cattaneo et al., 1988, 1989). We sequenced the central part of the haemagglutinin (H) gene coding region (nucleotides 588 to 1586) using primers MH1 and MH4, MH1 and MH6, MH2 and MH4, MH3 and MH5, and MH7 and MH5. Finally, we analysed nucleotides 1392 to 2283 in the fusion (F) gene, encoding the C-terminal 283 amino acids of the protein and including 41 nucleotides of the 3' untranslated region, using primers MF2 and MF1, MF3 and MF1, and MF4 and MF1. The C-terminal region of the F protein has previously been shown to contain premature stop codons producing truncated F proteins in some SSPE-associated measles virus isolates (Cattaneo et al., 1989).

Sequence analyses of these genomic regions (Fig. 1 to 4) revealed that all three isolates (TT, CL and SE) were different from each other and markedly different from the Schwarz vaccine strain used in the U.K., previously reported isolates, the sequences of which are related to vaccine strains (Edmonston, Hu2 and Hallé; Taylor et al., 1991), and SSPE isolates (cases I, A, B and C; Fig. 2 to 4 and Cattaneo et al., 1988, 1989). Isolate TT, obtained from a child with Kawasaki disease, was clearly different from isolates CL and SE, although they did share a number of changes which may be characteristic of viruses circulating in the U.K. in 1988 (see below). We did not observe any drastic alterations in isolate TT. In particular, there was no hypermutation of the M gene and the F gene did not contain the premature stop codons found in some SSPE isolates (Cattaneo et al., 1988, 1989). Therefore, within the limitations of this study, isolate TT
Fig. 1. Nucleotide sequence alignment of the region encoding the C terminus of NP. The predicted amino acid consensus sequence is shown underneath the nucleotide consensus sequence with amino acid changes shown in parentheses. An antibody epitope (Buckland et al., 1989) is underlined. TT, measles virus isolate from a child with Kawasaki disease; CL and SE, wild-type measles virus isolates obtained in 1988 (see text); SH, Schwarz vaccine strain; E, Edmonston vaccine strain (Rozenblatt et al., 1985); MVO, MVP, S33 and S81, U.K. wild-type isolates (see Taylor et al., 1991); IF(C), U.S.A. case (Cattaneo et al., 1989); CM, wild-type isolate, U.S.A. (Taylor et al., 1991); R118 and Y22, wild-type isolates, Africa (Giraudon et al., 1988; Taylor et al., 1991). The numbering is the same as that in Taylor et al. (1991).

seemed to exhibit the degree of variability expected from any U.K. isolate and was not related to vaccine strains. If there is indeed a link between some cases of Kawasaki disease and measles virus we can exclude an exclusive role of a vaccine or vaccine-related virus. As reverted mumps and polio attenuated vaccine viruses are known to be occasionally responsible for vaccine-associated disease, and as Kawasaki disease was first described at the time measles vaccination became widely used, this result is worth noting.

Isolates CL and SE, which were obtained from children who presented with 'classical' measles to the same General Practice within 3 weeks, were very closely related and differed only by one nucleotide change.

Fig. 2. Nucleotide sequence alignment of the M gene region analysed. See legend to Fig. 1 for the origin of isolates TT, CL, SE, SH; E sequence is from Bellini et al. (1986). Hu2, Schwarz vaccine-related isolate (Curran & Rima, 1988); 1 and B, SSPE cases (Cattaneo et al., 1988, 1989). The numbering is the same as in Curran & Rima (1988).
resulting in a change from a Met to a Thr codon in the M gene (position 885). This demonstrates the close relationship between measles viruses from a single outbreak. The identity of the NP gene region of these two isolates was confirmed by reamplifying and resequencing RNA from stored short-term PBMC cultures. Apart from excluding laboratory contamination as an explanation for the similarity of these two isolates, this finding also indicates that only a small number of changes (if any) occur during in vitro culture and passage in cell lines. This is in marked contrast to what has been observed, for example, for human immunodeficiency virus type 1 (Meyerhans et al., 1989; Vartanian et al., 1991).

In the NP gene region analysed, all three isolates had some changes in common, some of which had previously been noted to be typical of isolates from the U.K. (positions 1399, 1474, 1516 and 1551; see Taylor et al., 1991). In addition, our three isolates shared further common changes in the NP gene which are not present in the NP genes of other U.K. isolates (positions 1440, 1552, 1622 and 1656), but lacked two of the common changes in U.K. isolates (positions 1494 and 1563). This may reflect the evolution of circulating wild-type viruses in the U.K., given that all our isolates were made in 1988, whereas the other U.K. isolates (Taylor et al., 1991; Fig. 1) were either made in the mid-1970s or derived from patients presumably infected in the late 1970s or early 1980s (B. K. Rima, personal communication). None of these characteristic common changes found in the NP gene of our isolates was present in that of earlier U.S. isolates (Fig. 1; Taylor et al., 1991). In contrast, when we compared the H gene sequences of our isolates with those from two U.S. wild-type isolates from 1989 (sequences kindly provided by W. Bellini), we found that, with the exception of the U to C residue change at position 1088, every change common to the TT, CL and SE isolates was shared with these recent U.S. isolates. With one exception, none of the changes common only to CL and SE or unique to TT was present in these two U.S. strains. Therefore it seems that it would be rare to find H gene changes characteristic for either U.K. or U.S. isolates made in the late 1980s. It is possible that changes characteristic for different measles virus lineages, as identified by Taylor et al. (1991), are confined to the NP gene. However, these findings may also reflect an increasing mixing of measles viruses on a world-wide basis since the late 1970s, when the isolates analysed by Taylor et al. (1991) were in circulation. The comparison of the NP gene sequences from our 1988 U.K. isolates with those of Taylor et al. (1991) would also suggest that there has been significant evolution of measles viruses circulating in the U.K. over a period of about 10 years.

Regarding the relationship among vaccine-related isolates we noted some differences between positions
For defining lineage relationships between measles virus isolates we found the 3'-terminal region of the NP gene as well as the H gene most informative. The region of the NP gene analysed in this study encodes a B cell epitope (SRASDARAHLLPTGLPDID; Buckland et al., 1989), in which we observed an Ala to Val and a Gly to Asn change in isolates CL and SE, and a Gly to Ser change in isolate TT which is also present in this epitope of wild-type U.K. isolates. This epitope has previously been reported to be variable in some African strains of measles virus (Giraudon et al., 1988).

Within the protein region encoded by the H gene analysed in this study, antibody epitopes have also been shown to exist between amino acids 188 and 189 (Mäkelä et al., 1989a; Partidos et al., 1991) and 309 and 318 (Mäkelä et al., 1989b). We observed one amino acid change at position 316 (Gly to Ser) of isolates CL and SE, caused by a change at nucleotide position 966 (see Fig. 4). We also found a new possible glycosylation site in the H gene of isolates CL and SE at nucleotide position 1266 (Fig. 4). In isolate TT this site was shifted two amino acids downstream (nucleotide position 1273).

In summary, our study on the nucleotide sequence variability of a measles virus isolate from a child with Kawasaki disease in comparison to two contemporaneous wild-type isolates demonstrates that the former is not related to vaccine-related measles virus strains and gives an indication of the variability of measles viruses in the U.K. in 1988. By comparison with previously reported sequences the degree of sequence evolution which has occurred over the last decade was also demonstrated.

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**References**


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Fig. 4. Nucleotide sequence alignment of the H gene region analysed. See legend to Fig. 1 to 3 for the origin of isolates TT, CL, SE, SH, E, L, I and B. The numbering is the same as that in Gerald *et al.* (1986).


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