Development of an ELISA to detect MX virus, a human calicivirus in the Snow Mountain agent genogroup

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MX virus is a Snow Mountain agent (SMA) genogroup human calicivirus (HuCV) identified in a Mexican child with diarrhoea. An ELISA using hyperimmune antisera to the recombinant MX virus (rMX) capsid was developed to detect SMA genogroup HuCVs in stool specimens. The rMX ELISA detected the prototype MX virus, SMA, and Hawaii agent (HA), but not Norwalk virus (NV) or Sapporo virus. Twenty-three diarrhoea stool specimens from children attending day care centres in Norfolk, Virginia, were positive by the rMX ELISA and results were confirmed by reverse transcription-polymerase chain reaction (RT–PCR). Eight of 20 diarrhoea stool specimens from children in the United Kingdom previously shown to contain small round structured viruses (SRSVs) or HuCVs by electron microscopy were also positive by the rMX ELISA and RT–PCR. Sequence analysis of the RT–PCR products showed that all the rMX ELISA-positive viruses belong to the SMA genogroup. These data also showed that the SMA genogroup can be further divided into two subgroups: subgroup 1 includes prototypes SMA and HA, and subgroup 2 includes MX virus, mini-reovirus, Oth-25 and Bristol virus.

Introduction

The cloning and sequencing of the Norwalk virus (NV) genome (Jiang et al., 1990, 1993) has led to classification of NV in the calicivirus family and to the description of genomic sequences of several strains of human caliciviruses (HuCVs) (Ando et al., 1995; Cubitt et al., 1994; Green et al., 1993; Jiang et al., 1995a, b; Lambden et al., 1993; Lew et al., 1994a, b, c; Matson et al., 1989; Moe et al., 1994; Wang et al., 1994). Based on differences among the sequences of the RNA-dependent RNA polymerase region of the genomes of these strains, HuCVs have been classified into three genogroups: NV, SMA and Sapporo (Jiang et al., 1995a; Matson et al., 1995; Wang et al., 1994). The SMA genogroup viruses appear to have predominated in the last few years although NV and Sapporo genogroups have been detected (Jiang et al., 1995a; Wang et al., 1994; Ando et al., 1995; Cubitt et al., 1994). Several prototype HuCVs, such as HA and mini-reovirus, previously reported to be antigenically or morphologically distinct from SMA, now have been classified tentatively into the SMA genogroup (Lew et al., 1994a, b).

Although genetic relationships among many HuCV strains have been described, the antigenic relationships among and within the three HuCV genogroups remain to be elucidated. The major reason for this is the lack of suitable immunological tests for individual genogroups. Over the past 20 years, detection of HuCVs has relied on electron microscopy (EM), which is less sensitive, or on immunological tests which utilized reagents derived from clinical sources. The reagents have been in limited supply and have undefined specificity. Despite these limitations, the reagents have been used in immune EM (IEM) (Cubitt, 1985; Okada et al., 1990) or solid-phase IEM (SPIEM) (Lewis, 1990, 1991) to describe antigenic types of HuCV. The cloning of NV cDNAs resulted in development of a new generation of ELISAs which use hyperimmune antisera and viral antigens expressed in the baculovirus system for diagnosis of NV infection (Graham et al., 1994; Gray et al., 1993; Jiang et al., 1992a, 1995a; Numata et al., 1994; Parker et al., 1993, 1994; Treanor et al., 1993). The new assays enabled differentiation of the NV genogroup from the other two genogroups (Jiang et al., 1995a). Unfortunately, the wide genetic diversity of HuCVs requires development of
assays for antigenic typing of HuCVs not in the NV genogroup.

We recently cloned and expressed the viral capsid protein of MX virus (Jiang et al., 1995b, c). Sequence analysis of the RNA polymerase region of the MX genome showed that MX virus belongs to the SMA genogroup. The viral capsid protein of MX virus was expressed in a baculovirus recombinant and self assembled into virus-like particles (VLP) which morphologically, were similar to but antigenically distinct from, rNV VLPs (Jiang et al., 1992a, 1995c). An ELISA using the purified rMX VLPs detected specific seroresponses of volunteers infected with SMA and HA, suggesting that rMX virus is related antigenically to SMA (Jiang et al., 1995c). In this study, we describe the development of an ELISA using hyperimmune antisera to the rMX VLPs for detection of MX viral antigen in human stool specimens. We demonstrate that the rMX ELISA, like the rNV ELISA, is highly sensitive and specific and can be used for antigenic typing of SMA genogroup HuCVs.

**Methods**

**Stool specimens containing HuCVs.** The prototype MX virus from which we cloned the MX viral capsid gene was derived from a stool specimen from a Mexican child (Jiang et al., 1995b). Stool specimens from volunteers experimentally infected with NV (Graham et al., 1994) were supplied by Dr Mary K. Estes at Baylor College of Medicine. Eight stool specimens from volunteers infected with SMA and eight stool specimens from volunteers infected with HA were derived from previous challenge studies performed at the University of Rochester (Madore et al., 1990). Two HuCV Sapporo viruses were from a previous study (Matson et al., 1995); one from an outbreak of gastroenteritis in an orphanage in Sapporo, Japan in 1982 and the other from an outbreak of a child day care centre (DCC) in Houston, Texas, USA in 1986. Twenty stool specimens from children with acute gastroenteritis treated at the Hospital for Sick Children, London between 1989 and 1992, were obtained from the Institute of Child Health, London, UK (Cubitt et al., 1994). These 20 specimens contained SRSVs or morphologically typical HuCVs by EM examination and half of the specimens contained HuCVs by RT–PCR using primers NV36 and NV35 (Cubitt et al., 1994). Over 120 diarrhoea stool specimens from eight DCCs and 70 asymptomatic stool specimens from one DCC were obtained in our weekly surveillance of diarrhoea in child DCCs in Norfolk, Virginia, USA from November 1993 until November 1994.

**Immunization of laboratory animals with rMX antigen.** Hyperimmune antisera against MX virus were produced in rabbits and guinea-pigs by immunization with the baculovirus-expressed rMX capsid protein. Prior to immunization, the rMX viral capsid expressed in the insect Sf9 cells was purified by sucrose and CsCl gradient centrifugation (Jiang et al., 1995c), shown by electrophoresis to contain a single band of viral capsid protein and shown by EM to contain a homogeneous population of VLPs.

Preimmune sera were collected before the first injection of the immunogen. The immunization regimen included one intramuscular injection of the purified viral proteins in Freund’s complete adjuvant followed by two booster injections 2 weeks apart of the same dose in Freund’s incomplete adjuvant. The animals were bled 2 weeks after each booster injection and tested for antibody response to rMX (Jiang et al., 1995c). A final bleed was performed 2 weeks after the last booster injection, when the animal showed high antibody titres to rMX antigen.

**ELISA to detect MX antigen in stool.** A format similar to the assay for detection of NV antigen in human stools (Graham et al., 1994) was used to develop an ELISA for MX virus. Duplicate wells of a microtitre plate were coated with an optimal dilution (1:5000) of pre- or post-immunization rabbit antisera to rMX and the plates were incubated for 4 h at room temperature. The plates then were blocked with 5% Blotto in PBS for 1 h at 37 °C and washed twice with PBS containing 0.1% Tween-20 (PBS-TW). A 50 µl aliquot of test stool sample (10% suspension in 0.1% Blotto-PBS) was added to each well. After washing the plates four times with PBS-TW, hyperimmune guinea-pig anti-rMX serum (50 µl/well) was added at 1:5000 dilution in 1% Blotto-PBS and the plates were incubated for 2 h at 37 °C. The plates again were washed four times with PBS-TW and horseradish peroxidase (HRP) - conjugated goat anti-guinea-pig antiserum (Sigma) was added at a dilution of 1:5000. After another incubation for 2 h at 37 °C, the plates were washed a final four times with PBS-TW and 50 µl of the substrate 3,3’,5,5’-tetramethylbenzidine (TMB; Microwell Peroxidase Substrate System, Kirkegaard & Perry Laboratories) was added to each well. The plates were kept for 10 min at room temperature and the reaction was stopped and read at 450 nm in a TiterTek Multiskan ELISA reader (Tecan SLT Labinstruments). Each test plate included a negative and a positive control stool specimen with a known antigen titre.

**Detection of viruses in stool specimens by RT–PCR.** An RT–PCR technique (Jiang et al., 1992b) was used to detect HuCVs in stool specimens. Two primer pairs were used, each based upon the sequence of the RNA-dependent RNA polymerase region of the NV genome. Primers 36/35 were expected to produce a product of 470 bp, and primers 36/39 were expected to produce a product of 400 bp (Jiang et al., 1992; Wang et al., 1994).

The CTAB method (Jiang et al., 1992b) was used to extract RNA from stool specimens for RT–PCR. Reverse transcription was performed with slight modification from the previous description. In a 50 µl reaction, 5 µl of extracted RNA and 5 µl of avian myeloblastosis virus reverse transcriptase (Life Sciences) were mixed with 1 x PCR buffer (10 mm-Tris-HCl pH 8.3, 1.5 µg-MgCl2, 50 µM-KCl, 3.3 mM each of dATP, dCTP, dGTP and dTTP), 1.0 µM primer and 5 U of RNasin. The reaction was performed at 42 °C for 1 h. After incubation, 50 µl of 1 x PCR reaction mixture containing the second primer, and 5 U of Taq polymerase were added. The steps of the PCR cycling program were 94 °C for 1 min, 37 °C for 1 min and 72 °C for 90 seconds for 40 cycles.

**Sequence analysis of RT–PCR-amplified products.** RT–PCR-amplified products were cloned using the pGEMT vector (Promega) and the cloned inserts were sequenced using a sequencing kit from USB. The sequences of the cloned viral cDNAs were analysed using PC/GENE software, version 6.90 (IntelliGenetics). Multiple sequence alignment and a dendrogram indicating genetic relationships were generated using the CLUSTAL program, based on the method of Higgins & Sharp (1988).

**Results**

**Format of the rMX ELISA**

The hyperimmune guinea-pig and rabbit antisera had high antibody titres (> 1:500000) against the rMX capsid antigen. A sandwich ELISA using the two
hyperimmune antisera was developed to detect MX virus antigens in human stools. We found that the optimal format for the antigen-detection ELISA involved using the rabbit antiserum as coating antibody and the guinea-pig antiserum as detector antibody.

**Specificity of the rMX ELISA**

The specificity of the rMX ELISA was demonstrated by four methods. First, the rMX ELISA reacted strongly with rMX antigen expressed in the baculovirus system, but not with uninfected Sf9 cell lysates or rNV antigen (Fig. 1a). With the same amount of antigen in the assay, the rNV ELISA detected strong signals with rNV antigen but not with rMX or uninfected Sf9 cell lysates (Fig. 1b).

Second, we tested stool specimens from the Mexican child from whom we cloned the MX virus (Jiang et al., 1995b). Using stool specimens collected at weekly intervals from the child, strong signals were obtained when the child had diarrhoea and in the following week, but no reaction was observed in stool specimens collected before or two weeks after the clinical episode (Fig. 2).

Third, the MX virions in the diarrhoea stools of the Mexican child were purified by CsCl gradient centrifugation and tested in the rMX ELISA and by EM (Fig. 3). Two peaks of ELISA reactivity were observed in the gradient. The first peak occurred in fractions with a density of 1.38 g/cm³ (Fig. 3a) corresponding to full virions. Peak viral particle counts were observed by EM in these fractions (Fig. 3a). The second peak was in fractions with density of 1.31 g/cm³, corresponding to empty rMX viral particles derived from baculovirus (Fig. 3b).

Finally, we tested other human enteric viruses, including rotavirus serotypes 1 to 4, astrovirus serotypes 1 to 3, poliovirus I, ECHO virus 22, coxsackie virus, hepatitis A virus and enteric adenovirus types 40 and 41. None of these viruses were detected by the rMX ELISA.

**Detection of MX viruses in children attending DCCs**

To determine the cut-off point for the antigen ELISA, 60 stool specimens from healthy children not involved in an outbreak of gastroenteritis were tested. Fifty-eight (97%) of the samples had \( A_{450} \) values below 0.1 in wells coated with both the rabbit pre- and post-immunization antisera. Two samples had \( A_{450} \) values above 0.1 in both rabbit pre- and post-immunized antisera but the P/N (post-/preimmunization) values of the 2 specimens were < 2. The cut-off point was established as an \( A_{450} > 0.1 \) and a P/N > 2.

A total of 1200 diarrhoea stool specimens from children attending eight DCCs in Norfolk enrolled in our weekly surveillance study of diarrhoea between
Fig. 2. Detection of MX virus in stool specimens from the Mexican child from whom the MX virus was cloned by the rMX ELISA and RT–PCR. Stool specimens from the child before, during, and after the onset of acute gastroenteritis were tested. The titres of each sample were expressed by the reciprocal numbers of the dilution at which they were positive. The nature of the stools (watery or solid) are indicated at the top of the figure.

Fig. 3. CsCl gradient profiles (■) of MX viruses from stool (a) and rMX capsid (□) from baculovirus (b) as determined by rMX ELISA. The $A_{450}$ values of each sample in the wells coated with the rabbit post-immunization serum were plotted. The fractions from the stool sample also were examined by EM. The arrow heads indicate the fractions in which viral particles were identified by EM.
November 1993 and November 1994, were tested with the rMX ELISA. Twenty-four diarrhoea stool specimens from 21 children from five DCCs were positive. Twenty-three of the 24 positive specimens were confirmed by RT-PCR. One specimen was consistently positive by the rMX ELISA but was negative by the RT-PCR. Six of the 23 RT-PCR products were sequenced and proved to be from SMA genogroup viruses (see Table 2).

Sensitivity of the rMX ELISA

The sensitivity of the test was determined by testing serial dilutions of purified rMX antigen and stool specimens containing MX viruses. A minimum of 1.0 ng/ml of the purified rMX capsid was detected (Fig. 1 a), which was equivalent to the sensitivity of the rNV ELISA (Fig. 1 b). This amount of rMX viral capsid protein represented $10^6-10^7$ virions based on the estimation that each virion contains 180 molecules of capsid protein with a molecular mass of 57000 Da (Jiang et al., 1995c; Prasad et al., 1994).

With serial dilutions of stool specimens from the Mexican child infected with MX virus, the rMX ELISA was positive at a dilution of 1:128 of the stool specimen collected at the peak of the clinical episode of diarrhoea. The stool specimen collected 1 week later was positive at a stool dilution of 1:16. RT-PCR was slightly more sensitive, detecting viral RNA at dilutions of 1:170 and 1:20, respectively (Fig. 2).

Detection of prototype HuCVs by the rMX ELISA

To determine whether the rMX ELISA can be used for antigenic typing of HuCVs, prototype HuCVs representing the three known genogroups of HuCVs were tested in the assay. Two of 8 stool specimens from volunteers infected with SMA and 1 of 8 stool specimens from volunteers infected with HA were positive by the rMX ELISA, suggesting that SMA and HA are antigenically related to MX virus. Four stool specimens from volunteers infected with NV genogroup viruses (8FIIaNV/68) and 2 stool specimens containing Sapporo genogroup viruses (HuCV/Sapp/82 and HuCV/UT/86) were negative.

Detection of MX viruses by rMX ELISA in children with diarrhoea in the United Kingdom

Twenty diarrhoea stool specimens from children admitted to the Hospital for Sick Children in London were tested in the rNV and rMX ELISA. All 20 specimens were positive for small round structured viruses (SRSVs) or HuCVs by EM, but were negative by the rNV antigen ELISAs. Eight of the 20 specimens were positive by rMX ELISA (Table 1) and results were confirmed by RT-PCR. Four of the 8 RT-PCR products were cloned and sequenced (Cubitt et al., 1994) and all four revealed sequences close to SMA and MX virus (Table 2). Three stool specimens containing SRSVs were negative by rMX ELISA but positive by RT-PCR. The genetic identity of these viruses was not determined owing to insufficient materials necessary for cloning and sequencing.

Table 1. Detection of HuCVs in diarrhoeal stools of children from the UK by ELISA and RT-PCR

<table>
<thead>
<tr>
<th>Sample</th>
<th>A450 (pre/post)</th>
<th>P/N</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.051/0.055</td>
<td>1:1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>0.051/0.070</td>
<td>1:4</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.053/0.077</td>
<td>1:5</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0.064/0.272</td>
<td>4:3</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>0.070/0.090</td>
<td>1:3</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>0.078/0.083</td>
<td>1:1</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>0.065/0.226</td>
<td>3:5</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>0.049/0.075</td>
<td>1:5</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>0.061/0.145</td>
<td>2:4</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>0.050/0.194</td>
<td>3:9</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>0.076/0.228</td>
<td>3:0</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>0.048/0.228</td>
<td>4:8</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>0.049/0.057</td>
<td>1:2</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>0.029/0.232</td>
<td>8:0</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>0.062/0.048</td>
<td>0:8</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>0.059/0.061</td>
<td>1:0</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>0.058/0.223</td>
<td>3:8</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>0.013/0.048</td>
<td>3:7</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>0.056/0.074</td>
<td>1:3</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>0.076/0.056</td>
<td>0:7</td>
<td>-</td>
</tr>
</tbody>
</table>

* The RT-PCR products from these viruses were cloned and sequenced. They are SRSV/45/90/UK (#4), HuCV/3C/92/UK (#9), HuCV/5C/92/UK (#12), and HuCV/12C/92/UK (#17).
† This specimen was considered to be negative owing to the low A450 value of the well coated with the rabbit post serum, although the P/N ratio was over 2.
‡ Samples 9 and 10 were from the same patient and were both sequenced and found to be identical. Samples 1–7 had SRSV morphology and 8–20 HuCV morphology.

PCR. Four of the 8 RT-PCR products were cloned and sequenced (Cubitt et al., 1994) and all four revealed sequences close to SMA and MX virus (Table 2). Three stool specimens containing SRSVs were negative by rMX ELISA but positive by RT-PCR. The genetic identity of these viruses was not determined owing to insufficient materials necessary for cloning and sequencing.

Sequence analysis of the HuCVs detected by rMX ELISA

The sequences of the RT-PCR products of the newly detected viruses were compared with those of 3 prototype HuCVs and the MX virus (Table 2 and Fig. 4). Correlation between antigenic and genetic typings was observed. Viruses detected by the rMX ELISA showed high (> 83%) aa sequence identity with prototype SMA and MX viruses, but low aa sequence identity with NV genogroup (57–61%) and Sapporo genogroup (30–33%) HuCVs. None of the rMX ELISA-positive specimens were positive by the rNV ELISA and vice versa. Although high conservation was observed within antigenic groups of SMA genogroup viruses, significant
Table 2. Genetic and antigenic relationships of HuCVs determined by type-specific ELISAs and RT-PCR

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Amino acid similarity (%)</th>
<th>ELISA (+/tested)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NV</td>
<td>SMA</td>
<td>Sapp</td>
</tr>
<tr>
<td>8F11a NV/68/US†</td>
<td>100</td>
<td>57</td>
<td>30</td>
</tr>
<tr>
<td>SMA/76/US†</td>
<td>57</td>
<td>100</td>
<td>32</td>
</tr>
<tr>
<td>HA/71/US</td>
<td>61</td>
<td>91</td>
<td>33</td>
</tr>
<tr>
<td>MX 34/89/Mex†</td>
<td>58</td>
<td>89</td>
<td>30</td>
</tr>
<tr>
<td>SRSV/4S/90/UK</td>
<td>60</td>
<td>91</td>
<td>32</td>
</tr>
<tr>
<td>Nfk 1135/94/US‡</td>
<td>60</td>
<td>90</td>
<td>32</td>
</tr>
<tr>
<td>Nfk 1184/94/US‡</td>
<td>60</td>
<td>90</td>
<td>32</td>
</tr>
<tr>
<td>Nfk 770RI/94/US‡</td>
<td>60</td>
<td>90</td>
<td>32</td>
</tr>
<tr>
<td>HuCV/3C/92/UK</td>
<td>60</td>
<td>90</td>
<td>32</td>
</tr>
<tr>
<td>HuCV/5C/92/UK</td>
<td>60</td>
<td>90</td>
<td>31</td>
</tr>
<tr>
<td>Nfk 5230/94/US‡</td>
<td>59</td>
<td>89</td>
<td>30</td>
</tr>
<tr>
<td>Nfk 5432/94/US‡</td>
<td>58</td>
<td>88</td>
<td>30</td>
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<tr>
<td>Nfk 6610/94/US‡</td>
<td>60</td>
<td>93</td>
<td>32</td>
</tr>
<tr>
<td>HuCV/12C/92/UK</td>
<td>59</td>
<td>89</td>
<td>31</td>
</tr>
<tr>
<td>HuCV/Sapp/82/J†</td>
<td>30</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>HuCV UT/86/US</td>
<td>30</td>
<td>32</td>
<td>95</td>
</tr>
</tbody>
</table>

* Individual viruses are named by designated strain/year of isolation/country. The prefix 'HuCV' indicates those strains with typical caliciviral morphology, and prefix 'SRSV' indicates those strains without caliciviral morphology.
† Prototype NV, SMA, Sapporo, and MX viruses used as references in the sequence comparisons.
‡ Indicates viruses detected in the child DCCs in Norfolk, Virginia.

sequence variation was observed among viruses derived from different times and geographical locations (Fig. 4). Two potential subgroups of SMA genogroup were observed: subgroup 1 shared high sequence identity with SMA and subgroup 2 shared high sequence identity with MX virus. This subgrouping also was reflected in the results of testing these viruses in the rMX ELISA. The SMA and HA prototypes belong to subgroup 1 and a low (19%) rate of detection was obtained when stool specimens from volunteers infected with SMA and HA were tested with rMX ELISA.

Discussion

This study describes an ELISA using hyperimmune antisera against the baculovirus-expressed rMX viral capsid protein to detect SMA genogroup HuCVs in stool specimens. Like the rNV ELISA, the rMX ELISA is simple, sensitive, and suitable for large clinical and epidemiological studies of HuCV-associated illness. Because the rMX antigen ELISA detected SMA genogroup but not NV- and Sapporo genogroup HuCVs, it is useful for antigenic typing of SMA genogroup strains.

We previously reported that HuCVs can be divided into at least three genogroups: NV-, SMA- and Sapporo genogroups (Jiang et al., 1995a; Matson et al., 1995; Wang et al., 1994). In this study, we report that the SMA genogroup can be divided further into two subgroups based upon sequence differences in the RNA polymerase region of the genomes. Subgroup 1 includes the prototype SMA and HA, and subgroup 2 includes MX virus, minireovirus, Oth-25 and Bristol virus. Similar profiles of sequence variation of HuCVs from different clinical specimens were also observed by others (Ando et al., 1995). We use the term 'subgroup' because: (i) strains within the subgroups are clustered genetically and are predicted to be equidistant from the NV- and Sapporo genogroups and (ii) the subgroups are related antigenically as determined by the rMX antibody (Jiang et al., 1995c) and antigen ELISAs, although the antigen ELISA reacted differently with the two subgroups. Viruses in the MX-subgroup reacted strongly in the MX antigen ELISA, and viruses in the SMA-subgroup reacted weakly, which corresponded with the low detection rates for stool specimens from volunteers infected with SMA and HA. The monoclonal antibody specific for SMA (Treanor et al., 1988) did not react with the rMX antigen. In conclusion, genetic variation within the SMA genogroup was associated with antigenic variation which suggested the existence of two subgroups within the genogroup. This variation exhibited by the current rMX ELISA would be potentiated by ELISAs utilizing monoclonal antibody specific for two subgroups.

Based upon reactivities in the rNV and the rMX ELISAs, we predict that HuCVs can be divided into three antigenic groups similar to the three genogroups described above. This classification scheme remains preliminary because many strains genetically classified in the NV genogroup, such as KY89 and SRSV3, and SMA...
Fig. 4. Dendrogram of the RNA polymerase region of 22 HuCVs. The prefix ‘HuCV’ indicates strains with typical calicivirus morphology, and the prefix ‘SRSV’ indicates strains without typical calicivirus morphology as described in the footnote of Table 2. The amino acid sequences between primers 36/39 (~386 bases) in the RNA polymerase region of the viral genomes were aligned using the PC/GENE multiple alignment program. The length of each horizontal line indicates the genetic distance between the viruses normalized to 1.0 for the dendrogram. The stars indicate viruses which were tested and positive in the rMX ELISA.

genogroup, such as minireovirus, Oth-25, and Bristol virus, remain to be tested. The Sapporo genogroup was clearly distinct from NV- and SMA genogroups by the rNV and rMX ELISAs, however, type-specific assays for this genogroup remain to be developed. In addition, three antigenic types were described in the US (Wyatt et al., 1974; Dolin et al., 1982), four serotypes were reported in the UK (Lewis, 1990, 1991) and up to nine serotypes were found in Japan (Okada et al., 1990). A worldwide collaboration utilizing a single test will be necessary to clarify the antigenic classification of HuCVs.

In comparison to the classification schemes based upon antigenic reactivities, the genetic classification method appears to be more reliable. The ELISAs utilizing the baculovirus-derived reagents also are highly specific and reproducible. The correspondence of antigenic and genetic groups observed in this study further confirms the reliability of the new methods. IEM was the major technique for antigenic typing of HuCVs before the cloning of NV. One weakness of observations using this technique is that serum specimens from clinical sources were utilized. Because the exposure history to
different strains of HuCVs of patients is unknown, the specificity of the antibody in the patient's serum is not reliable. In addition, the quantities of test sera have been limited, which makes it difficult to perform direct comparison studies across the world. The antisera derived from the baculovirus antigen are of known specificity and of unlimited supply. One difference between our observations and those of previous reports is that HA, previously found to be antigenically distinct from SMA by IEM and cross-challenge studies (Wyatt et al., 1974; Dolin et al., 1982), now would be classified in the same subgroup with SMA. We believe these differences will be resolved when new reagents are used.

This study is the first to describe SMA genogroup infections and associated illness among children using both antigenic detection and RT-PCR. The finding of both SMA subgroups in the UK and the US suggested that the SMA genogroup is common in these countries. This has been confirmed by seroprevalence studies in both countries measuring antibody to the rMX capsid (Parker et al., 1995 and our own unpublished observations). The present study suggests that the SMA genogroup currently is more prevalent than the NV genogroup in the US and the UK. Another study showed that SMA genogroup can be traced to the early 1980s in the UK (D. Cubitt & X. Jiang, unpublished results). Although no systematic survey has yet been conducted, accumulated reports indicate that the SMA genogroup is currently predominant in many countries, including Japan (Jiang et al., 1995a; Wang et al., 1994), Canada (Lew et al., 1994), the UK (Cubitt et al., 1994; Green et al., 1993), Mexico (Jiang et al., 1995b), South Africa (M. Wolfaardt, M.B. Taylor, W.O.K. Grabow, W.D. Cubitt & X. Jiang, unpublished results) and the US (Ando et al., 1995; Jiang et al., 1995a; Moe et al., 1994). Outbreaks of gastroenteritis associated with subgroup 1 strains in the SMA genogroup have also been reported recently in nursing homes for the elderly and at health department clinics in Virginia (X. Jiang et al., unpublished results). Further epidemiological studies using the new rMX ELISA are needed to understand the role of the SMA genogroup as a cause of illness in humans.

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