Antigenic properties of human parechovirus 1

Paivi Joki-Korpela,1,2 Merja Roivainen,3 Hilkka Lankinen,1 Tuija Pöyry1 and Timo Hyypiä1,2

1 Haartman Institute, Department of Virology, PO Box 21, University of Helsinki, FIN-00014 Helsinki, Finland
2 Department of Virology and MediCity Research Laboratories, University of Turku, FIN-20520 Turku, Finland
3 National Public Health Institute, Enterovirus Laboratory, Mannerheimintie 166, FIN-00300, Helsinki, Finland

Introduction

Human parechoviruses (HPEVs) belong to the family Picornaviridae together with aphtho-, cardio-, entero-, hepato- and rhinoviruses. HPEV1 and HPEV2 were previously named echoviruses 22 and 23, respectively, and classified among the entroviruses. Molecular analysis has revealed that the genomic sequences of HPEVs differ remarkably from those of other picornaviruses and this has led to establishment of a new genus, parechoviruses (Hyypiä et al., 1992; Stanway et al., 1994; Ghazi et al., 1998; Oberste et al., 1998; Stanway & Hyypiä, 1999). Protein processing of HPEV1 was also shown to be different from that of other previously studied picornaviruses, since the maturation cleavage of capsid protein precursor VP0 to VP2 and VP4 is not seen in HPEV1 (Stanway et al., 1994). Growth properties of HPEVs in cell culture appear to differ from those of enteroviruses; this was observed soon after HPEVs were isolated (Wigand & Sabin, 1961; Shaver et al., 1961; Jamison, 1974). It has been shown that HPEV1 infection is highly common (Sato et al., 1972; Grist et al., 1978; Joki-Korpela & Hyypiä, 1998): for example, in a Finnish population studied, 97% of adults were seropositive, and the antibodies appeared early in life (Joki-Korpela & Hyypiä, 1998). A third candidate for the parechovirus genus is Ljungan virus, a new picornavirus isolated from bank voles in Sweden which shows considerably high sequence identity with HPEVs (Niklasson et al., 1999).

To study the immunological properties of this new picornavirus genus, we carried out an antigenic analysis of HPEV1 capsid proteins. One specific object of interest was the region containing the tripeptide motif arginine–glycine–aspartic acid (RGD) located near the C terminus of the capsid protein VP1. The RGD-containing extension to the VP1 capsid polypeptide of coxsackievirus A9 (CAV9; an enterovirus) has previously been shown to be antigenic (Pulli et al., 1998a, b). Such motifs are known to participate in cell–cell and cell–matrix interactions (Ruoslahti & Pierschbacher, 1987) and are also utilized by several viral pathogens in their attachment and entry. It has been shown that the RGD-recognizing integrin αvβ3 functions as a receptor for CAV9 and that HPEV1 competes with CAV9 for cell surface binding (Roivainen et al., 1994). Another aim of the study was to recognize immunogenic peptides located elsewhere in the capsid which could be used, for instance, as antigens in HPEV-specific serological assays.

Human parechoviruses 1 and 2 (HPEV1 and HPEV2, respectively), formerly known as echoviruses 22 and 23, have been assigned to a novel picornavirus genus on the basis of their distinct molecular and biological properties. To study the immunological characteristics of HPEV1 capsid proteins, antigenic analysis was carried out by a peptide scanning technique, which can be used to identify the immunogenic peptide sequences of a protein. Partially overlapping peptides, representing the capsid of HPEV1, were synthesized using a 12 aa window in a three residue shift and reactivity of rabbit and murine HPEV1 antisera against these peptides were tested. Using this method, an antigenic site in the VP0 polypeptide, recognized by both rabbit and murine antisera, was identified. The sequence of this region was conserved among HPEV1 clinical isolates obtained from Finland and the United States. Antiserum against this peptide region showed neutralizing activity against HPEV1 in cell culture. Because the C-terminal region of HPEV1 VP1 contains a functional RGD motif, the antigenicity of this region was also tested. By using the corresponding peptide antiserum, neutralization of HPEV1 was observed. Cross-neutralization between HPEV1 and coxsackievirus A9, an enterovirus with a similar RGD motif in VP1, was also detected.
Methods

**Viruses, cells and clinical isolates.** CAV9 (Griggs strain) and coxsackievirus B4 (CVB4) (J.V. B.) were obtained from the ATCC. The infectious HPEV1 (Harris) cDNA clone and CAV9 RGD deletion mutants (Hughes et al., 1995) were kindly provided by Glyn Stanway, Department of Biological Sciences, University of Essex, Colchester, UK. The A549 (human lung carcinoma) cell line (ATCC) was used for transfection of the RNA transcript, virus culture and neutralization tests. The GMK (green monkey kidney) cell line was used for CAV9 and CVB4 neutralization assays. HPEV1 isolates for sequence analysis were obtained from the Department of Virology, University of Turku, Finland, from the Department of Virology, Haartman Institute, University of Helsinki, Finland, and from Mark Pallansch at the Centers for Disease Control and Prevention, Atlanta, GA, USA. The viruses from Finland were isolated in Turku in 1991 and in Helsinki in 1995, whereas the isolates from USA were obtained from Colorado (1973), Georgia (1979) and Missouri (1992).

**Antigens and antibodies.** HPEV1, CAV9 and CVB4 antisera were obtained by immunizing rabbits and mice with virus purified in sucrose gradients (Abraham & Colombo, 1984). Rabbits were immunized with three sequential 20–100 µg doses injected at 2- to 4-week intervals using the popliteal lymph-node method (Leinonen, 1985), including the use of Freund’s complete adjuvant in the first dose. The sera were collected 2 weeks after the last dose. Mice were immunized subcutaneously with three sequential doses of purified virus at 2-week intervals. Freund’s complete adjuvant was used at the first dose. The peptides used in immunoassays and for the production of the antisera were synthesized in an Applied Biosystems 433A peptide synthesizer using Fmoc chemistry. They were purified in reverse phase liquid chromatography and analysed for correct molecular mass in a matrix-assisted laser desorption ionization/time-of-flight mass spectrometer (FinneganMatt). Peptides P208 and P209 were selected on the basis of peptide scanning results, whereas P151 was chosen for this study because it contained the RGD region. Peptide P154 (VP0 N terminus) was used as a negative control for HPEV1 and CAV9 antisera as this region was not reactive against HPEV1 rabbit antisera in peptide scanning. In HPEVs, there is an apparent N-terminal extension to the VP3 capsid protein which is not seen in other picornaviruses and peptide P152, which represents this region, was therefore chosen for this study.

**Peptide scanning.** Partially overlapping peptides covering the HPEV1 capsid were synthesized in 96-spot format onto cellulose membranes (Frank, 1992) according to the manufacturer’s instructions (SPOTS; Genosys). The whole amino acid sequences of the capsid proteins VP0, VP3 and VP1 were scanned using a 12 aa window moved in three residue shifts. A method previously described for CAV9 (Pulli et al., 1998a) was used. The membranes containing the HPEV1 peptides were incubated overnight in blocking buffer (Genosys) and rinsed once with TBS–Tween (Tris-based saline; pH 8.0, 0.05 % Tween 20). After this, the membranes were incubated for 30 min with rabbit or mouse antisera diluted 1:1000 in the blocking buffer. The membranes were washed 5 × 5 min with TBS–Tween, and incubated for 30 min with peroxidase-conjugated swine anti-rabbit or rabbit anti-mouse immunoglobulin (Dako) diluted 1:1500 in the blocking buffer. After washing again 5 × 5 min with TBS–Tween, the membranes were incubated for 1 min in ECL detection reagent (Amersham). Chemiluminescence was counted in a 1450 MicroBeta liquid scintillation counter (Wallac) for 1 s per spot. The final results were calculated as an average of the four spots where the same amino acid residue was present. The results were also visualized by exposing the membranes for 1 min on an X-ray film. The visual results correlated well with the LCPS (luminescence counts per s).

The membranes were regenerated by treating them 5 × 10 min in 8 M urea/1% SDS/0.1% β-mercaptoethanol followed by 5 × 10 min in 50% ethanol/10% acetic acid. Finally, the membranes were washed 3 × 5 min with TBS–Tween and stored at 4 °C.

**Reverse transcription, PCR and sequence analysis.** RNA was isolated from clinical samples using the Ultraspec RNA isolation system (Biotex Laboratories). The RNA pellet was dissolved in water and stored at −70 °C until analysed. The primers used for reverse transcription, PCR and sequence analysis amplified a 490 nt region from the VP0 capsid protein gene. The C1 + (5′ AAAACGTCATGTTGGCC 3′) primer represented HPEV1 genomic RNA (Hyppiä et al., 1992) at positions 445–462, whereas the C2 – (5′ GGTARTTCNACATGTAATCTG 3′) primer was complementary to positions 1113–1135. We also used primers from the VP1 capsid protein gene of HPEV1, which amplify a 374 nt fragment. The C3 + (5′ TTTCCACATATGACAC 3′) primer was of genomic polarity (positions 2722–2741) and the C4 – (5′ GTARAAAACCTCATCTAAATA 3′) primer was complementary to the sequence at positions 3076–3096. cDNA synthesis was performed (37 °C for 1 h) in a reaction mixture (40 µl) containing 5 µl specimen RNA, 8 µl 5 × RT buffer (Promega), 2 µl dNTP mix (10 mM dATP, dCTP, dGTP, dTTP; Boehringer Mannheim), 0.1 µl ribonuclease inhibitor RNasin (40 U/µl; Promega), 50 pmol C2 – or C4 – primer and 0.1 µl MMLV reverse transcriptase (200 U/µl; Promega). For PCR, 5 µl cDNA reaction mixture was combined with 75 µl water, 10 µl 10 × PCR buffer (Finnzymes), 2 µl dNTP mix (10 mM dATP, dCTP, dGTP, dTTP; Boehringer Mannheim), 0.5 µl Taq DNA polymerase (2 U/ml; Finnzymes) and 50 pmol primers (C1 +/C2 – or C3 +/C4 –). The mixture was amplified through 40 cycles of heating at 95 °C for 2 min, primer annealing at 54 °C for 2 min and DNA synthesis at 72 °C for 4 min. The PCR products were analysed in 1% agarose gels containing ethidium bromide. For sequencing, the PCR amplicons of expected size were purified from the agarose gels using the Qiaex II Gel Extraction kit (Qiagen) and sequenced with fluorescent dye-labelled terminators using an automated DNA sequencer (310 Genetic Analyser; Abi Prism). Primers C1 + and C3 + were used for sequencing.

**Plaque neutralization test.** A549 and GMK cells were grown to full confluency in 6-well cell culture dishes (Costar). Sixty microlitres of each antisera dilution in Hank’s balanced salt solution supplemented with 0.6 % foetal calf serum (dilution medium) containing approximately 100 p.f.u. HPEV1 was incubated for 1 h at 37 °C. The cells were washed once with Hank’s solution and the antisera–virus mixture was added. After incubation for 15 min at room temperature, the cells were overlaid with 0.5 % carboxymethyl cellulose in the cell culture medium (MEM) and the plaques were incubated in 5 % CO2 atmosphere at 37 °C for 48 h. After this, the medium was removed and the cells were stained with crystal violet solution prior to counting the number of plaques. For the CAV9 and the RGD mutants, 50 µl virus diluted 1:100 in dilution medium was mixed with an equal volume of a 1:4 dilution of HPEV1 or CAV9 antisera. The mixtures were incubated for 2 h at 37 °C and diluted for subsequent infection for GMK cells from 10-3 to 10-8. The cells (GMK) were infected and stained as above.

**Enzyme immunoassay (EIA) and immunoblotting.** Ninety-six-well microtitre plates (Maxisorp; Nalge Nunc International) were coated at room temperature overnight with peptides (5 µg/ml in 50 mM NaHCO3; pH 9.6; 150 µl per well) or with purified HPEV1 or CAV9 (0.3 µg/ml, 50 µl per well). The wells were washed three times with PBS–TWEEN (containing 0.1 % Tween 20) and blocked with 0.1 % BSA in PBS (300 µl per well) for 30 min. The wells were washed as above and sera diluted in PBS supplemented with 1 % BSA, 1 % foetal calf serum and 0.1 % Tween 20 were added into the wells. The plates were incubated for 1 h at 37 °C and washed three times with PBS–TWEEN. Horseradish...
peroxidase (HRP)-conjugated goat-anti rabbit IgG (1:10000 dilution; ICN Biomedicals) was added to the wells and the plates were incubated at 37 °C for 1 h. After washing as above, the H₂O₂/o-phenylenediamide substrate/chromogen solution was added and the reaction was stopped after 30 min with 1 M H₂SO₄. The absorbance was measured at 492 nm in a spectrophotometer (Multiscan EX; Labsystems). Proteins in lysates of HPEV1-, CAV9- or CBV4-infected cells were separated by SDS–PAGE and blotted onto nitrocellulose membranes (Schleicher and Schuell) which were subsequently incubated in PBS containing 5% milk powder (Valio) (PBS–milk) for 15 min at room temperature to block non-specific binding. The HPEV1 antiserum was diluted 1:1000, CAV9 antiserum diluted 1:1000, CBV4 antiserum diluted 1:100 and P151 peptide antiserum diluted 1:100 in PBS–milk and, after overnight incubation at room temperature with the antiserum, the membranes were washed as above in PBS–Tween (containing 0.05% Tween 20). Visualization was achieved by HRP-conjugated anti-rabbit serum (Bio-Rad Laboratories).

**Results**

**Peptide scanning**

In order to identify B-cell epitopes recognized by rabbit and murine immune systems in HPEV1, we used the peptide...
Table 1. Amino acid sequences and locations of the synthetic peptides used in the study

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence</th>
<th>Location (aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P154</td>
<td>METIKSIADMATGV</td>
<td>N terminus of HPEV1 VP0 (1–12)</td>
</tr>
<tr>
<td>P208</td>
<td>NLTQHPSAPTMP</td>
<td>N-terminal region of HPEV1 VP0 (79–90)</td>
</tr>
<tr>
<td>P209</td>
<td>TMPFSPDFSVNVD</td>
<td>N-terminal region of HPEV1 VP0 (88–99)</td>
</tr>
<tr>
<td>P152</td>
<td>NGKKKKWKKIM</td>
<td>N terminus of HPEV1 VP3 (3–13)</td>
</tr>
<tr>
<td>P151</td>
<td>SRALRGDMANLTNQ</td>
<td>C terminus of HPEV1 VP1 (218–231)</td>
</tr>
</tbody>
</table>

scanning technique where partially overlapping peptides covering the viral capsid polypeptides were synthesized. The tests were carried out using immune serum from a rabbit immunized with HPEV1 and a serum pool of three HPEV1-immunized mice. Rabbit preimmune serum and pooled sera from three non-immunized mice were used as controls.

Rabbit immune serum recognized clearly one linear immunogenic site in the N-terminal part of the VP0 capsid protein (aa 82–99) (Fig. 1). The mouse immune serum pool reacted with an overlapping antigenic region (aa 79–90) as well as with another region at the C-terminal part of the VP0 polypeptide (aa 253–271). Two regions in the VP3 capsid protein were reactive with the rabbit antiserum. One site was located in the N-terminal part of the polypeptide (aa 15–35) and the other one was localized in the C-terminal region (aa 183–195). The N terminus of the VP3 capsid protein (aa 2–14), adjacent to the antigenic site recognized by the rabbit antiserum, showed some reactivity with the murine antiserum pool. The rabbit antiserum did not recognize any clearly reactive antigenic regions in the VP1 polypeptide, although the RGD-containing region in the C-terminal region (aa 218–231) showed weak reactivity which exceeded the background.

Enzyme immunoassay and immunoblotting

To further study the antigenicity of peptides corresponding to the major antigenic region identified by peptide scanning and other selected peptides from the capsid proteins, we tested the reactivity of different rabbit antisera by EIA. Microtitre plates were coated with the synthetic peptides or purified virus and the binding of sera from rabbits immunized either with purified virus or peptides was tested. The sequences of the peptides used in the experiments are shown in Table 1.

Peptides P208 and P209, covering the antigenic region located by peptide scanning in the HPEV1 capsid protein VP0, were recognized by the two HPEV1 rabbit immune sera and by the corresponding peptide antiserum, whereas no significant reactivity was seen with the corresponding preimmune sera (Fig. 2). The two HPEV1 rabbit immune sera reacted with the purified HPEV1 but, somewhat surprisingly, none of the sera raised against HPEV1 peptides recognized the virus on the
Fig. 3. Reactivity of rabbit antisera against HPEV1, CAV9 and synthetic peptides in EIA against purified HPEV1, P151 (C terminus of VP1) and P154 (N terminus of VP0). Symbols for immune and preimmune sera: HPEV1 no. 1 (+ -), HPEV1 no. 2 (U -V), CAV9 ( - -), P154 (-- E --, -- D --), P151 (-- _ --, -- ^ --).

Solid phase (Fig. 3). However, immune serum against another RGD-containing picornavirus, CAV9, showed some binding to HPEV1. Cross-reactivity in CAV9-coated wells was also observed when HPEV1 immune sera were tested (data not shown). However, no cross-reactivity was observed in immunoblotting when antisera prepared against purified HPEV1, CAV9, CBV4 and peptide P151 were assayed against lysates of the cells infected with the three viruses (data not shown). P151, a 14 aa RGD-containing peptide from the HPEV1 VP1 capsid protein, was recognized efficiently by HPEV1 rabbit hyperimmune sera, but gave only weak reactivity with the corresponding peptide antiserum (Fig. 3). The antiserum against peptide P154, representing the extreme N terminus of HPEV1 VP0, showed reactivity with the corresponding peptide, and both HPEV1 and CAV9 rabbit immune sera reacted with this peptide in low dilutions (Fig. 3).

Neutralization test

We also tested whether rabbit antisera raised against different synthetic peptides (Table 1) contained neutralizing antibodies against HPEV1. Antiserum dilutions of 1:30 (Fig. 4) and 1:100 (not shown) were used in this assay. When compared to the preimmune serum, rabbits immunized with peptides representing the RGD-containing region in the HPEV1 VP1 capsid protein and the immunogenic region located in the VP0 capsid protein were the most efficient ones for production of neutralizing antibodies. When a dilution of 1:30 was used, the antiserum raised against the peptide containing the RGD region was able to neutralize 51% of HPEV1 infectivity, whereas preimmune serum blocked only 1% of virus infectivity (27% and 9% in the 1:100 dilution, respectively). Antiserum raised against the peptides P208 and P209, covering the antigenic site in VP0, each neutralized 43% of HPEV1 infectivity, whereas the corresponding figures with preimmune sera were 18% and 13% (Fig. 4). The two immune sera together were able to neutralize 76% of HPEV1 infectivity in cell culture, whereas the blocking effect of combined preimmune sera was 33%. In other peptide antisera tested in 1:30 dilution, the difference in neutralization was less than 12% when immune and preimmune serum were compared.

We also studied the immunological cross-neutralizing activity of antisera against HPEV1, CAV9 and CBV4. HPEV1 rabbit immune serum, as well as CAV9 immune serum, completely inhibited HPEV1 infection in cell cultures at
Table 2. Cross-neutralization activity (%) of rabbit antisera raised against HPEV1, CAV9 and CBV4

Dilutions 1:30 and 1:100 were used. HPEV1 antiserum neutralized HPEV1 at a dilution of 1:10^5 and CAV9 antiserum neutralized CAV9 at a dilution of 1:10^3.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Dilution</th>
<th>HPEV1</th>
<th>CAV9</th>
<th>CBV4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPEV1</td>
<td>1:30</td>
<td>100</td>
<td>95</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>100</td>
<td>35</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>CAV9</td>
<td>1:30</td>
<td>100</td>
<td>100</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>100</td>
<td>100</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>CBV4</td>
<td>1:30</td>
<td>30</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Neutralization of CAV9 and RGD-mutants (Hughes et al., 1995) with HPEV1 and CAV9 rabbit immune sera in GMK cells

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sequence (VP1 C-terminus)</th>
<th>p.f.u./ml × 10^6</th>
<th>No anti-serum</th>
<th>HPEV1 anti-serum</th>
<th>CAV9 anti-serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAV9</td>
<td>TTVAQSSRRGDMSTLN</td>
<td>280</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CAV9-m4</td>
<td>TTVAQSSR---MSTLN</td>
<td>3.9</td>
<td>2.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CAV9-m12</td>
<td>TTV----------LN</td>
<td>1.4</td>
<td>1.4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Recently, a new picornavirus genus, Parechovirus, was established. This was due to the unique molecular and biological properties of the type member, HPEV1 (previously known as echovirus 22) (Stanway & Hyypia, 1999). Earlier studies have shown that HPEV1 is a common human pathogen and that infection is usually experienced during the first years of life (Sato et al., 1972; Grist et al., 1978; Joki-Korpela & Hyypia, 1998). HPEV1 causes, in most cases, infections of the gastrointestinal or respiratory tract (Stanway et al., 2000). When compared, for instance, to enterovirus infections, the involvement of the central nervous system is less frequent (Grist et al., 1978), although cases ofencephalitis and paralysis have been reported (Koskiniemi et al., 1989; Figueroa et al., 1989). Myocarditis, necrotizing enterocolitis and haemolytic uremic syndrome have also been associated with HPEV1 infection (Maller et al., 1967; Russell & Bell, 1970).

Although immunogenic epitopes of some representatives of picornaviruses have been relatively well characterized, little is known about the detailed antigenic determinants of most of the members of the family. In order to elucidate the antigenic properties of HPEV1, we applied various techniques to study
the immunogenicity of the virus and its cross-reactivity with RGD-containing CAV9 and CBV4, which has no RGD motif. By using the peptide scanning technique, an immunodominant epitope was recognized in the N-terminal region of the VP0 capsid polypeptide (positions 79–99). When compared with picornaviruses whose three-dimensional virus particle structure is known (e.g. Hogle et al., 1985; Rossmann et al., 1985; Luo et al., 1987; Acharya et al., 1989), this region seems to follow the βA2 sheet and by analogy with these viruses, it is predicted to form a loop structure buried between VP2 and VP3 (Stanway et al., 1994). It is possible, however, that such buried sequences can become exposed to the immune system, as previously shown for a highly immunogenic region in the poliovirus VP1 protein (Roivainen et al., 1993). Rabbit antibodies raised against the peptide covering the antigenic HPEV1 VP0 sequence inhibited infection partially in cell culture. In sequence comparisons of clinical HPEV1 isolates and the reference strain, this site was highly conserved, and was even closely related to the animal parechovirus-like agent, Ljungan virus. Again, an analogous situation occurs in enteroviruses, where a highly conserved region near to the N terminus of VP1 (Hovi & Roivainen, 1993) is reactive in peptide scanning (Roivainen et al., 1991a; Hovi & Roivainen, 1993; Pulli et al., 1998a).

HPEV1 has an RGD tripeptide at the C terminus of capsid protein VP1 and the infection can be blocked by RGD-containing peptides (Stanway et al., 1994). The corresponding region has been shown to be functionally important and antigenic in CAV9, an enterovirus with a similar RGD motif in a similar position (Roivainen et al., 1991a; Chang et al., 1992). The reactivity of the C-terminal part in HPEV1 VP1 was weak in peptide scanning. However, when EIA was used, rabbit hyperimmune serum against HPEV1 reacted strongly with the RGD-containing peptide. This phenomenon could be due to the different conformation of the epitope when different
Antigenic regions of picornaviruses have been previously studied by using synthetic peptides, peptide scanning and neutralization escape mutants. In poliovirus 1 (PV1), PV3, CAV9, human rhinovirus 14 (HRV14) and FMDV, the major antigenic regions frequently contain amino acid sequences from the capsid protein VP1, whereas VP2 and VP3 are less often involved in the antigenic structures (Minor et al., 1986; Sherry et al., 1986; Roivainen et al., 1991b; Usherwood & Nash, 1995; Pulli et al., 1998a). In light of these results, HPEV1 capsid protein VP1 was surprisingly poorly reactive in peptide scanning and the localization of the major antigenic region in VP0 differs from most of the other picornaviruses studied. As a method, peptide scanning is ideal for mapping linear epitopes, but discontinuous antigenic sites can also be identified by this method (Roivainen et al., 1991b). However, a negative result in peptide scanning does not exclude the possibility, that the studied region is immunogenic, especially when strictly conformational epitopes are concerned.

The antigenic region identified in HPEV1 is located approximately 25 aa after the site corresponding to VP2/VP4 cleavage in enteroviruses, whereas the major antigenic regions described previously for other picornaviruses (e.g. PV1, CAV9, FMDV, HRV2, HRV14) are situated mainly in the B–C and E–F loops of VP2 (Rossmann et al., 1985; Minor et al., 1986; Sherry et al., 1986; Appleyard et al., 1990; Mateu, 1995; Pulli et al., 1998a). In Theiler’s murine encephalomyelitis virus, a picornavirus causing demyelinating disease in mice, a predominant antigenic region at the N terminus of VP2 (aa 2–14) appears to be more close to the VP4/VP2 cleavage site than the HPEV1 antigenic region (Kim et al., 1992). Detailed structural data of HPEV1 particles would be helpful in understanding these differences.

In conclusion, our studies revealed an immunodominant epitope in the VP0 polypeptide, in a region which is not found to be antigenic in other previously studied picornaviruses. Both rabbit and mouse immune systems recognized this antigenic region when the peptide scanning technique was used. Remarkable conservation of this region was observed when sequences obtained from clinical specimens from different geographic locations were compared. Although the C-terminal region of CAV9 VP1, which also contained the functionally analogous RGD motif, has been shown to be highly immunogenic by peptide scanning (Pulli et al., 1998a, b), no significant reactivity was seen in this region in HPEV1. However, the RGD-containing peptide was antigenic when ELA was used and this peptide was able to induce production of neutralizing antibodies in a rabbit. Also, this region was relatively well conserved among HPEV1 strains. The antigenic cross-reactivity between HPEV1 and CAV9 suggests that cross-neutralization between picornaviruses sharing only short common epitopic sequences can occur.

We wish to thank Dr Glyn Stanway and Dr Tapani Hovi for stimulating discussions during the preparation of manuscript and Dr Stanway and Dr Pamela Hughes for the HPEV1 cDNA clone and CAV9 mutants. The skilful technical assistance of Ms Mervi Eskelinen is greatly acknowledged. The study was supported by grants from the Academy of Finland, the Sigrid Juselius Foundation and Helsinki Graduate School of Biomedical Sciences.

References


an RGD motif in VP1 is functionally significant. *Journal of General Virology* 73, 621–626.


Received 19 October 1999; Accepted 14 March 2000