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Monoclonal antibody against VP0 recognizes a broad range of human parechoviruses

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ABSTRACT

Parechoviruses (PeVs) are common viruses that cause mild gastrointestinal or respiratory symptoms to severe central nervous system infections. In infants, parechovirus infection is one of the leading causes of life-threatening viral disease. High-quality antibodies with broad binding specificities are essential to improve accurate parechovirus diagnosis in diagnostic laboratories. Such antibodies have potential in the development of rapid antigen detection assay against PeVs. In the present study, VP4 and VP2 genes from human parechovirus A1 (PeV-A1) were cloned and VP0 fusion protein produced to develop monoclonal antibodies against PeVs. Two pan-parechovirus antibodies, one IgG and one IgM isotype, were isolated. The properties of IgG1/κ monoclonal (designated as Mab-PAR-1) was studied further. Mab-PAR-1 was shown to be functional in western blot against denatured recombinant protein and viral particles. In immunofluorescence assay, the antibody tested positive for nineteen PeV-A1 isolates while showing no cross-reactivity to fourteen entero- and rhinovirus types. In addition, Mab-PAR-1 showed positive reactivity against five other cultivable parechovirus types 2–6. A unique Mab-PAR-1 epitope located in the junction of the three capsid proteins VP0, VP1, and VP3 was identified using a peptide library screen. This study demonstrates that PeV-A1-VP0 protein is functional antigen for developing monoclonal antibody for diagnosis of broad range of parechovirus infections.

1. Introduction

Human parechoviruses (PeVs), are small, positive-sense, single-stranded RNA viruses in the genus Parechovirus within *Picornaviridae* family (de Crom et al., 2016). PeVs are mainly transmitted via faeco-oral or respiratory routes (Harvala and Simmonds, 2009). Currently, there are nineteen distinct PeV types, but not all are known to cause severe disease and only half of the types have been cultivated in experimental cell models. Majority of PeV infections affect children and are either asymptomatic or cause mild respiratory or gastrointestinal illnesses (Harvala and Simmonds, 2009; Kadambari et al., 2019). Severe complications arise only in minority of infected children, predominantly caused by PeV-A3 (Ghanem-Zoubi et al., 2013; Han et al., 2013; Schuffenecker et al., 2012; Sedmak et al., 2010; Selvarangan et al., 2011), and may lead to myocarditis, encephalitis, pneumonia, meningitis or flaccid paralysis (Esposito et al., 2014; Harvala et al., 2014). Several studies indicate that PeV-A infection of the central nervous system (CNS) can affect normal neuro-development leading to long-term sequelae, such as speech impairment, motor abnormalities, visual impairment or cerebral palsy (Britton et al., 2018).

Diagnosis of PeV infections depends on laboratory testing since their clinical symptoms vary and overlap with other disease agents. Laboratory diagnosis of PeV-A infection is currently by reverse transcription quantitative polymerase chain reaction (RT-qPCR) (Benschop et al., 2008; Renaud et al., 2011). While PCR methods including multiplex RT-PCR have led to rapid diagnosis of PeV-As, they are far from the reach of populations in economically poor and developing nations. The lack of broadly specific antibodies for the detection of PeVs may be due to high antigenic diversity of PeV capsids, and thus there are no specific antibodies currently available for their detection. However, serological assay development has suggested that there are conserved regions in the parechoviral capsids, which may be functional immunogenic sites to develop specific monoclonal antibodies (Abed et al., 2007; Alho et al., 2003; Yu et al., 2012).

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In this work, we developed a pan-parechovirus (Mab-PAR-1) mouse monoclonal antibody using GST-tagged PeV-A1-VP0 protein as an antigen. We analyzed the reactivity of the developed Mab-PAR-1 antibody against different PeV-A1 isolates as well as other cultivable PeV types using immunofluorescence assay. Furthermore, the antibody epitope was identified using a peptide library generated by using PeV-A1-VP0 protein (Harris strain) as a template. To our knowledge, this is the first monoclonal antibody described for broad detection of human parechovirus types.

2. Materials and methods

2.1. Viruses and cell lines

PeV-A1 (Harris) and PeV-A2 (Williamson) prototype strains were purchased from ATCC. PeV-A1 (152478, 452252 and 350757, PeV-A3 (152037 and K251181-02), PeV-A4 (K251176) and PeV-A5 (20552322) isolates were kind gifts from Dr. Katja Wolthers (AMC, Amsterdam, the Netherlands). PeV-A1 153-20, 101-17, 103-2, 125-7, 145-12, and 150-8 were from National Institute of Health and Welfare (Helsinki, Finland). PeV-A1 isolates FI0003, FI0007, FI0008, FI0111, FI0114, FI0219, FI0222, FI0433, FI0435, FI0578 and PeV-A6 types (FI0147 and FI0189) were from our laboratory collections (Kolehmainen et al., 2012). Enteroviruses (CVA9, CVB2, CVB3, CVB5, E2, E11, E20 and E30) and rhinoviruses (RV-A1b, RV-A10, RV-A24, RV-B14, RV-B35 and RV-B86) were from ATCC. Virus lysates were prepared in A549, HeLa, HT-29 and RD cell lines, which were purchased from ATCC and maintained in Dulbecco's Modified Eagle medium (DMEM) supplemented with gentamicin and 10 % Fetal Bovine Serum (FBS).

2.2. Production and purification of antigens

Recombinant GST-PeV-A1-VP0 fusion protein and GST protein used as a control were purified as previously described (Alho et al., 2003) with minor modifications. Briefly, cell pellets were suspended and lysed in Bugbuster buffer (#70584-4, Novagen, USA) for 30 min on ice. Bugbuster buffer was supplemented with 2 mg/mL lysozyme (#L6876, Sigma, USA), Benzonase (#B16012, Biotool, USA) and protease inhibitor (#A32963, Thermo-Fischer, USA). The supernatant was separated by centrifugation at 10000 rpm for 10 min at 4°C. Supernatant was collected and incubated with glutathione sepharose (#GE17-0756-01, GE Healthcare, USA) in a column for 2 h at room temperature. After extensive washing, column elution was done with 20 mM Glutathione (#6200202, Bio-Rad, USA) prepared in PBS buffer, pH8.0. The eluates were collected and buffer was exchanged to PBS by dialysis overnight at 4°C. The purity of eluted protein fractions was analyzed with SDS-PAGE.

2.3. Hybridoma preparation and production of Mab-PAR-1

Two Balb/c mice were immunized with 20 µg of PeV-A1-VP0 recombinant protein in FCA adjuvant. Four booster injections were given in two weeks' intervals. Mice were sacrificed two days after final injection and splenocytes were fused to mouse myeloma cells using ClonaCell HY Kit (Stemcell Technologies, Canada). One thousand (1000) cell clones were cultivated on 96-well plates and tested for PeV-A1-VP0 on ELISA and immunofluorescence assay (IFA) in PeV-A1 (Harris strain) infected HT-29 cells. Twelve (12) clones were selected for further assaying of which one IgG and one IgM clone specific for PeV-A1 was isolated. IgG clone was chosen for further characterization. Antibody from this clone was isotyped as IgG1 κ and named Mab-PAR-1. Mab-PAR-1 was purified in CIM r-Protein G column (BIA Separations, Slovenia) using Äkta FPLC (GE Healthcare, USA). Protein purity was determined using SDS-PAGE and concentration was determined by BCA assay.

2.4. Western blotting

PeV-A1 infected or non-infected A549 cell-lysate, purified VP0-GST fusion protein and purified GST protein were prepared individually with SDS sample buffer and run in 4–20 % SDS PAGE (4561093S, Bio-Rad, USA). Page ruler pre-stained (#PI26619, Thermo Scientific, USA) was used as a protein size ladder. Separated proteins were transferred to nitro-cellulose membrane (#10600001, Life Science, USA). The blotted membranes were blocked overnight with TBS containing 5% BSA followed by blotting with 10 µg/mL Mab-PAR-1 in 1 % BSA in TBST for 1 h. After washes with TBST, membrane was incubated with anti-mouse IRDye® 680RD (#926-68070, LI-COR Biosciences, USA) for 1 h followed by washes and analyzing with Odyssey (LI-COR, USA).

2.5. Capture ELISA

Wells of 96-microtiter plate (#3590, Corning, USA) were coated with Mab-PAR-1 (500 ng/well) in bicarbonate buffer (100 mM, pH 9.6) and incubated overnight at 4 °C. Next day, the wells were washed with PBS-T buffer (PBS supplemented with 0.05 % Tween 20, pH 7.4) and blocked with 3 % BSA at room temperature for two hours. After three washes with PBS-T buffer, PeV-A1- or mock-inoculated cell lysates were pipetted onto wells coated with Mab-PAR-1. Purified VP0-GST and GST proteins were used as positive and negative controls, respectively. After one-hour incubation at room temperature, wells were washed four times with PBS-T buffer and rabbit anti-PeV-A1 polyclonal serum was added to the wells. After one-hour incubation, wells were washed four times with PBS-T and HRP-conjugated Goat anti-rabbit IgG antibodies (#111-035-003, Jackson Immunoresearch, USA) were added and kept at room-temperature for one hour. Wells were washed three times with PBS-T buffer, TMB substrate (#34028, Thermo-Fisher, USA) was added, and reaction was stopped with addition of 0.45 M sulphuric acid. OD450 values were measured with Victor^3 multilabel counter (PerkinElmer, USA).

2.6. Immunofluorescence assay

A549 or HT-29 cells seeded onto Viewplate-96 Black Clear Bottom plates (#6005182, PerkinElmer, USA) were infected with different dilutions of PeV-A isolates or types aiming to achieve 20–40 % infection per cell count in the well. The infection was allowed to proceed for six hours at 37 °C in incubator and 5 % CO_2. After six hours of infection, the cells were washed once with sterile PBS. Fixing of cells was carried out using 4% formalin and permeabilization with 0.2 % Triton-X100. Staining with Mab-PAR-1 antibody (1 µg/mL), Pan-Enterovirus Reagent (#3365, Light Diagnostics, USA) or mouse pan-entero monoclonal antibody (#C01700 M, Meridian, USA) in 3 % BSA in PBS at room temperature for one hour. After incubation and washing, cells were stained with Alexa Fluor 488-labelled anti-rabbit (#A-11008, life-technologies, USA) or anti-mouse (#A-11001, Life-technologies, USA) secondary antibody for one hour at room temperature. The nuclei were stained with DAPI (#D1306, Sigma, USA). EVOS FL-Auto was used to visualize the cells.

2.7. Epitope mapping

Epitope mapping was performed at Pepscan Presto BV (Lelystad, The Netherlands) using Pepscan's proprietary Chemically Linked Peptides on Scaffolds (CLIPS) technology. 15-mer peptides from the VP0 protein (corresponding to nucleotides 710–1576 of PeV-A1 Harris strain (GenBank acc no L02971)) were generated with an offset of one amino acid. Mab-PAR-1 antibody was incubated with the peptides immobilized on a glass slide (peptide library) in 4 % horse serum, 5 % ovalbumin (w/v) in PBS/1% Tween. After washing, the peptide library was incubated with a 1/1000 dilution of rabbit anti-mouse IgG(H + L) HRP conjugate for one hour at 25 °C, washed again, and incubated with the peroxidase

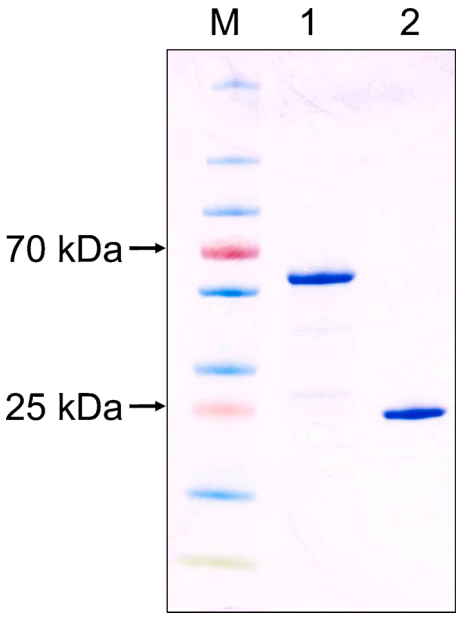

Fig. 1. SDS-PAGE analysis. Purified VP0-GST protein (lane 1) and GST protein used as a control (lane 2). The arrows indicate the positions of 25 and 70 kDa molecular weight markers (M).

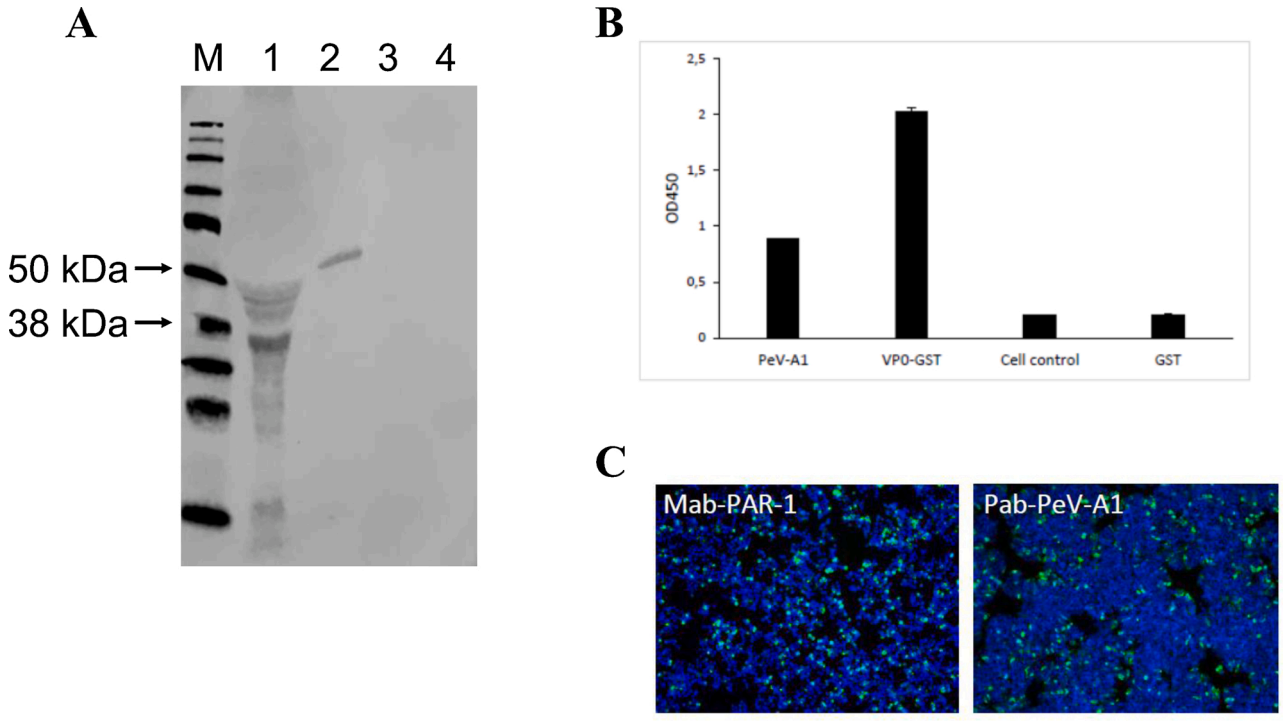

Fig. 2. Detection of PeV-A1-VP0 protein by Western blot (A) and ELISA (B). (A) The sizes of viral VP0 and recombinant VP0-GST fusion proteins are ~32 kDa and ~58 kDa, respectively. Samples were run on SDS-PAGE gel, transferred to nitrocellulose membrane and Western blot was carried out using rabbit raised GST antisera. Molecular weight marker (M), PeV-A1 infected (lane 1), VP0-GST fusion protein (lane 2), uninfected cell lysate (lane 3), GST protein (lane 4). **(B)** Capture ELISA assay was performed using Mab-PAR-1 (500 ng/well) as the capture antibody. Wells were coated with the antibody to capture the virus or VP0-GST protein. PeV-A1 infected or uninfected cell lysates, VP0-GST or GST were used as analytes, and in-house rabbit anti-PeV-A1 antiserum (Pab-PeV-A1) and HRP-conjugated goat anti-rabbit antibody were used as primary and secondary antibodies, respectively. Signals (OD450) were measured with Victor^3 multilabel counter. **(C)** Immunofluorescence assay with Mab-PAR-1 and Pab-PeV-A1. HT-29 cells were infected with PeV-A1 (Harris strain), fixed with paraformaldehyde, permeabilized six hours post-infection, followed by staining with Mab-PAR-1 antibody or Pab-PeV-A1 antiserum. Alexa 488-conjugated anti-mouse or anti-rabbit antibodies were used as secondary antibodies. Positive green fluorescent signals were visible only in infected cells. DAPI stained nuclei are visible in blue color. Stained cells were visualized using EVOS-FL Auto imaging microscope (10X magnification) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

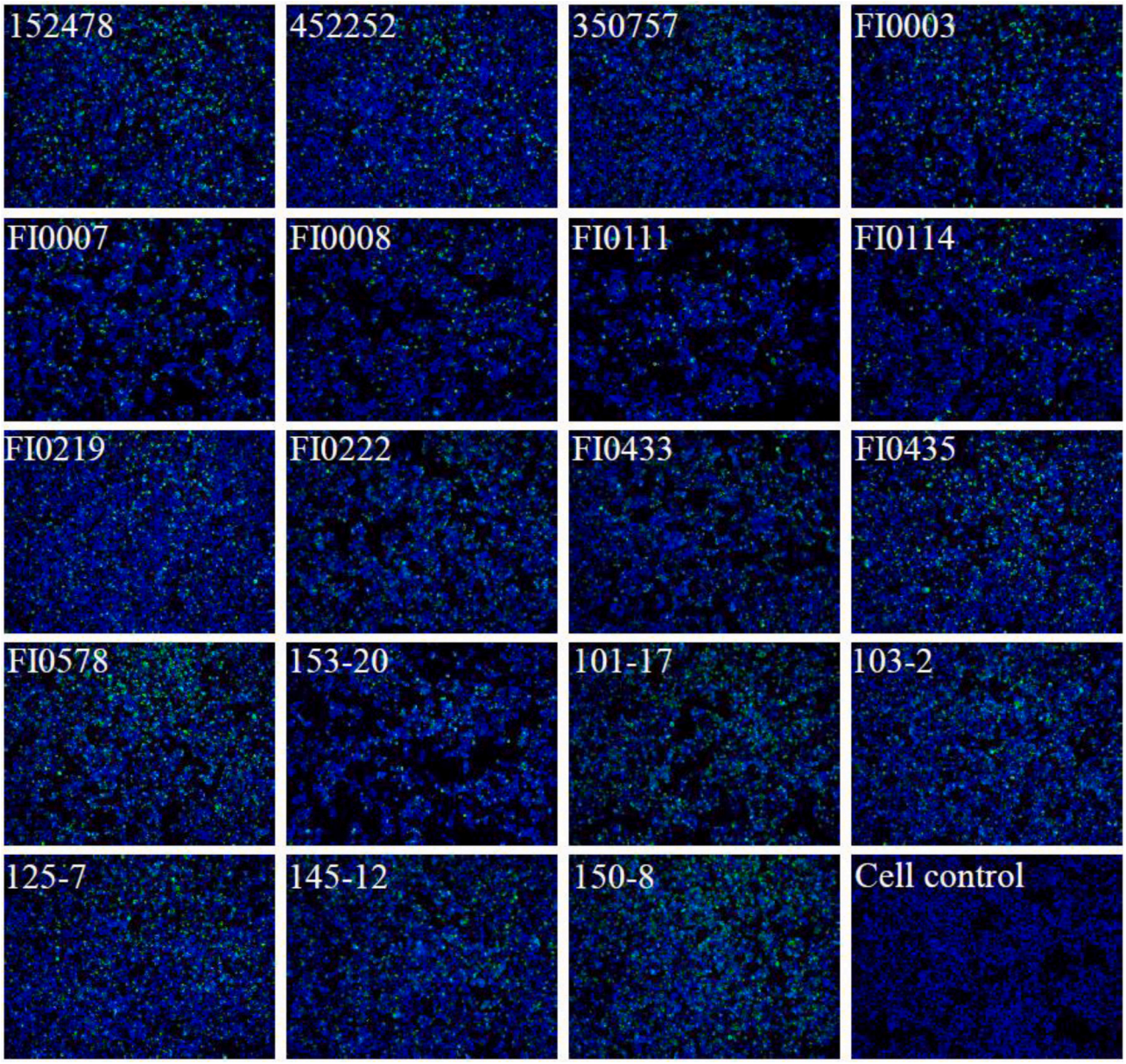

Fig. 3. Detection of PeV-A1-infected HT-29 cells with Mab-PAR-1. The HT-29 cells were either mock-infected or infected with 19 clinical PeV-A1 isolates followed by staining with Mab-PAR-1 antibody. Stained cells were visualized using EVOS FL Auto imaging microscope (10 × magnification). Numbers on top of each image indicate the codes for different clinical PeV-A1 isolates.

substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 µl of 3 % H_2O_2. After one hour, the color development was measured and quantified with a charge coupled device (CCD) camera and an image processing system (Slootstra et al., 1996).

2.8. Structural analyses

Structural analysis of the epitope was carried out using UCSF Chimera v1.15 (Pettersen et al., 2004). The structure of human parechovirus 1 virion obtained from the Protein Data Bank (PDB ID: 4z92) (Berman et al., 2000; Kalynych et al., 2016) was used as a reference to determine the exact location and surface exposure of the epitope.

3. Results

3.1. Preparation of PeV-A1-VP0-GST antigen and generation of hybridomas

To prepare a functional parechoviral immunogen, we chose to use VP0 protein, which is a fusion of VP4 and VP2 capsid proteins. PeV-A1-VP0 protein-encoding gene fragment was PCR-amplified and subcloned into pGEX-4T-1 vector. GST-tagged VP0 proteins were expressed in *E. coli* (BL21 cells), purified from soluble fraction by affinity chromatography using glutathione-sepharose beads, and analyzed by SDS-PAGE and Western blotting. Purified GST-tagged VP0 and GST proteins migrated between 55 and 70 kDa and close to 25 kDa markers on SDS-PAGE gel, respectively, which were consistent with their predicted molecular weights (58 kDa for VP0 and 25 kDa for GST) (Fig. 1).

To determine whether PeV-A1-VP0 is functional immunogen for monoclonal antibody development, two *Balb/c* mice were immunized

Table 1
Visualization of parechovirus A1 isolates and some entero- and rhinoviruses by Mab-PAR-1 in immunofluorescence microscopy assay. (+) positive detection, (-) no detection.

Virus tested	Strain	MAB-PAR-1 reactivity
PeV-A1	152478	+
PeV-A1	452252	+
PeV-A1	350757	+
PeV-A1	FI0003	+
PeV-A1	FI0007	+
PeV-A1	FI0008	+
PeV-A1	FI0111	+
PeV-A1	FI0114	+
PeV-A1	FI0219	+
PeV-A1	FI0222	+
PeV-A1	FI0433	+
PeV-A1	FI0435	+
PeV-A1	FI0578	+
PeV-A1	153-20	+
PeV-A1	101-17	+
PeV-A1	103-2	+
PeV-A1	125-7	+
PeV-A1	145-12	+
PeV-A1	150-8	+
HPeV-1	Harris	+
HPeV-2	Williamson	+
HPeV-3	152037	+
HPeV-3	K251181-02	+
HPeV-4	K251176	+
HPeV-5	20552322	+
HPeV-6	FI0147	+
HPeV-6	FI0189	+
CVA9	Griggs	–
CVB2	Ohio-1	–
CVB3	Nancy	–
CVB5	Faulkner	–
E2	Cornelis	–
E11	Silva	–
E20	JV-1	–
E30	Bastianni	–
RV-A1b		–
RV-A10		–
RV-A24		–
RV-B14		–
RV-B35		–
RV-B86		–

with 20 μg/dose of purified antigen mixed with FCA adjuvant. After four booster doses, a mouse with the best preliminary antibody titer (data not shown) was sacrificed and used for preparation of hybridomas. In total of one thousand antibody-producing monoclonal cell clones were cultivated and binding of unpurified antibody preparations were screened in small-scale against PeV-A1 virus using IFA assay. Surprisingly, only two PeV-A1-specific clones were identified. Isotyping of Protein A column-purified antibody preparations revealed that there were one IgG1/κ and one IgM clone (data not shown). IgG1/κ antibody was used in further experiments to study its specificity and binding properties.

3.2. Functionality of Mab-PAR-1 in Western blot analysis, capture ELISA and immunofluorescence assay against PeV-A1 virus and PeV-A1-VP0 protein

To characterize the properties of Mab-PAR-1, the antibody was tested against PeV-A1 Harris prototype strain (PeV-A1) and PeV-A1-VP0-GST protein in Western blot, capture ELISA and immunofluorescence assay (IFA). Western blot analysis was performed using cell lysate prepared from PeV-A1-infected A549 cells and purified VP0 protein. Uninfected cell lysate and GST protein were used as negative controls. As shown in Fig. 2, Mab-PAR-1 bound to proteins of ~32 KDa (lane-1) and 58 kDa (lane-2) in size, which correspond to the sizes of viral PeV-A1 VP0 and recombinant VP0-GST proteins. No signal was detected in the control lanes. Capture ELISA performed with PeV-A1-infected or uninfected A549 cell lysates indicated that Mab-PAR-1 binds to native virion structures (Fig. 2B). Purified Mab-PAR-1 and in-house produced rabbit anti-PeV-A1 polyclonal antiserum were used as coating and detection antibodies, respectively. The ability of Mab-PAR-1 to bind to PeV-A1-infected cells was further demonstrated by using HT-29 cells and IFA assay (Fig. 2C). The presence of fluorescence signals only in some cells indicate specific binding to PeV-A1 in infected cells.

3.3. Detection of PeV-A1 isolates, PeV types and related entero- and rhinoviruses by immunofluorescence assay

PeV-A1 is the most widely distributed and prevalent of all parechovirus types in different countries under national enterovirus surveillance programs (Benschop et al., 2011; Chiang et al., 2017; Khetsuriani et al., 2006; Van Der Sanden et al., 2013). Partial genome sequencing and subsequent phylogenetic analyses have indicated the presence of multiple PeV-A1 strains (Ito et al., 2010; Van Der Sanden et al., 2008). Since Mab-PAR-1 was developed against PeV-A1-VP0 protein, it was logical to assess its binding to different PeV-A1 isolates in IFA assay. Mab-PAR-1 antibody reacted positively with nineteen PeV-A1 clinical isolates inoculated onto HT-29 cells in 96-well plate (Fig. 3). To further evaluate the broad range specificity of Mab-PAR-1, binding to different cultivable PeV-A types was also analyzed (Table 1, Fig. 4). The results indicate that Mab-PAR-1 has broad specificity to six parechovirus type indicating that these viruses possess a highly conserved immunogenic epitope. Further analysis of antibody properties included testing ability to neutralize virus infection, since it is already known that PeV-A-VP0 elicits neutralizing antibody response (Alho et al., 2003). Pab-PeV-A1 antiserum was used as positive control and it was able to neutralize PeV-A1 with high serum dilution titer (160–10240), whereas, no neutralization was observed with Mab-PAR-1 antibody (data not shown).

To demonstrate that Mab-PAR-1 binds specifically to human parechoviruses but not related entero- or rhinoviruses, the cross-reactivity of the antibody was tested against eight enterovirus (EV) and six rhinovirus (RV) types; RD cells, a cell-line known to be permissive to EVs, was infected with eight different EV prototypes (CVA9, CVB2, CVB3, CVB5, E2, E11, E20 and E30). In parallel, HeLa cells were infected with six RV prototypes (RV-A1b, RV-A10, RV-A24, RV-B14, RV-B35 and RV-B86). All tested EVs and RVs gave positive signals with pan-EV antibodies, but no fluorescence signal was observed with Mab-PAR-1 antibody. Thus, no cross-reactions with entero- and rhinoviruses were detected with Mab-PAR-1 in IFA assay. Table 1 summarizes the findings. As a summary, Mab-PAR-1 is highly specific to human parechoviruses with no cross-reactivity to entero- and rhinoviruses.

3.4. Identification of Mab-PAR-1- epitope

To identify the epitope for Mab-PAR-1, a 15-mer peptide library was generated using PeV-A1-VP0 protein as a model. The Pepscan's CLIPS analysis identified a single epitope, VVTYDSKL, positioned at 192–199 amino acid region of PeV-A1-VP0. Structural analysis of the PeV capsid protein carried out in UCSF Chimera v1.15 (Pettersen et al., 2004) using PeV-A1 (Harris strain) crystal structure (PDB ID: 4z92) revealed the location of VVTYDSKL on the surface of the capsid (Fig. 5A). Interestingly, the epitope positions itself in the junction of the three capsid proteins VP0, VP1, and VP3 (Fig. 5B). Furthermore, the epitope exhibits significant surface exposure with 7 out of 8 residues being accessible from the cytoplasmic side of the capsid through the helix and loop structures present (Fig. 5C).

4. Discussion

To generate high-quality antibodies to human parechoviruses, we generated recombinant VP0 protein antigen using the sequence of

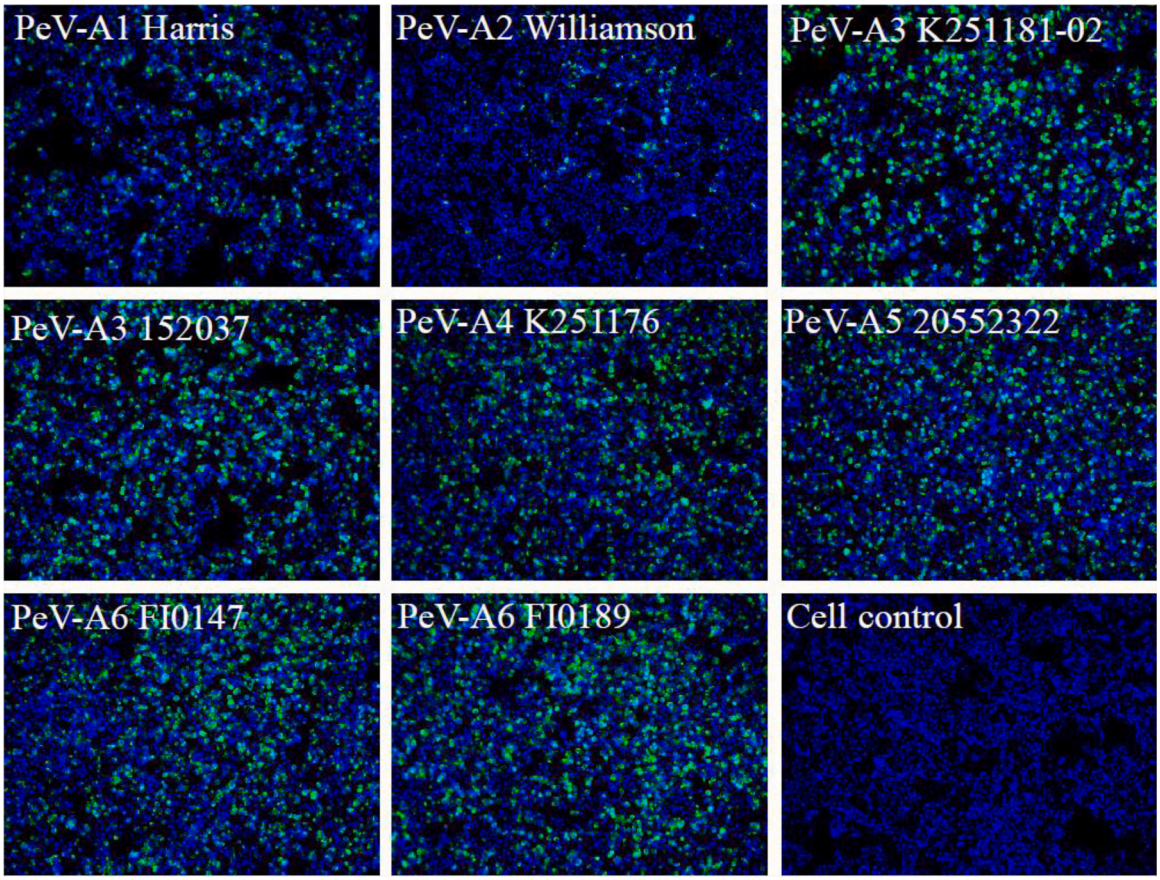

Fig. 4. Detection of PeV types by Mab-PAR-1 in IFA assay. The HT-29 cells were either mock-infected or infected with six PeV types followed by staining with Mab-PAR-1 antibody. Stained cells were visualized using EVOS FL Auto imaging microscope (10 × magnification). Numbers on top of each image indicate the code for the type.

human parechovirus A1 (PeV-A1) Harris strain (Fig. 1). The antigen was used to generate conventional hybridoma cell lines from which we isolated a single IgG1/κ-secreting clone among one thousand clones that were screened in the study. We successfully characterized the binding properties of Mab-PAR-1 against the prototype PeV-A1 virus and recombinant VP0 protein by Western blot, capture ELISA and IFA (Fig. 2). We further assessed the properties of Mab-PAR-1 against PeV-A1 clinical isolates and different cultivable PeV-A types (PeV-A2 to A6). We found that the Mab-PAR-1 monoclonal recognizes PeV-A1 clinical isolates and different PeV-A types with high specificity (Figs. 2 and 3) since no cross-reactivities were detected against different entero- and rhinoviruses (Table 1). Additional experiments allowed the identification of a unique antibody epitope on the surface of PeV-A1 capsid. The data indicate that we have generated and identified the first (to our knowledge) monoclonal antibody that broadly recognizes human parechovirus types. Use of parechoviral proteins as antigens have been described in a few serological papers (Abed et al., 2007; Alho et al., 2003; Yu et al., 2012), but until now there have been no reports of immunogenic sites within parechoviruses that could be used to generate broad detector antibodies.

Worldwide burden of PeV-As is not well documented since the virus has gone undiagnosed mainly due to lack of cheap diagnostic measures. The current state of the art method for parechovirus diagnosis is RT-qPCR, which is expensive and only applicable in developed countries. Consequently, most of the epidemic reports arise from developed nations, like USA (Selvarangan et al., 2011), Japan (Ito et al., 2010), Australia (Britton et al., 2018) or countries in European Union (Schuffenecker et al., 2012; Van Der Sanden et al., 2013). However, recent results from developing countries support the worldwide presence and distribution of PeV-As (Brouwer et al., 2019; Chiang et al., 2017; Han et al., 2013; Itta et al., 2017). At present, nineteen PeV-A types have been documented of which PeV-A1 and PeV-A3 are the most prevalent types. Both virus types have been linked to severe CNS-associated infections in infants (Ferreras Antolín et al., 2018), and thus their detection would be essential among other CNS-related pathogens. However, half of the types have only been characterized at type level, while the other half has been successfully cultivated in experimental cell cultures. Nevertheless, there is limited cellular research carried out even with cultivable human parechoviruses since there are no antibody tools for their detection. Development of the first highly specific antibodies against human parechoviruses is likely to potentiate cellular work.

Currently, there are no rapid assays employing specific antibodies against human picornaviruses including human parechoviruses. This is mainly due to high number of heterogenous types, which has made it difficult to identify conserved epitopes despite the accumulating number of full-length viral sequences and high-resolution structures that can be used for identification of such sites. To our knowledge, we present the first parechoviral antibody that has broad specifity to parechovirus types. Thus, suggest that PeV-A1-VP0 possesses a highly conserved immunogenic epitope. We anticipate that Mab-PAR-1 antibody has strong diagnostic potential against PeV-As.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

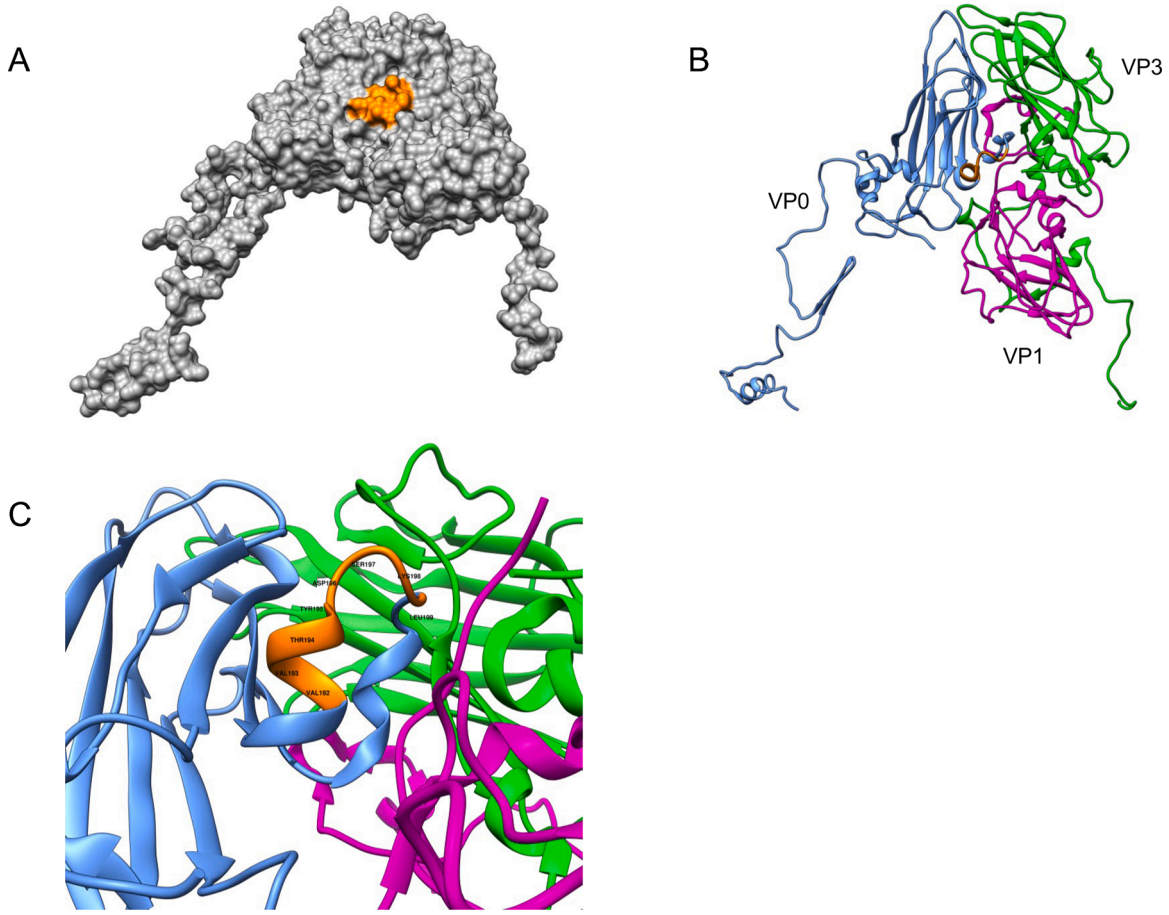

Fig. 5. Surface and structural representation of Mab-PAR1- epitope, VVTYDSKL. (A) The VVTYDSKL epitope marked on the surface of PeV-A1 with orange (PDB ID: 4z92). The epitope is seen with significant surface exposure on the cytoplasmic side of the capsid. (B) Ribbon representation of the PeV-A1 asymmetric. The VP0 protein is colored in blue, VP1 protein is colored in magenta, and VP1 protein is colored in green. The VVTYDSKL epitope is colored in orange. (C) A closer view of the VVTYDSKL epitope. Located in the VP0 protein, and in the junction of all three capsid proteins, the epitope is seen gaining significant surface exposure through the helical loop structure (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2021.114167.

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