Saffold cardiovirus infection in a 2-year-old boy with acute pancreatitis

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Summary

Saffold cardiovirus (SAFV) identified from a stool sample in 2007 is thought to be associated with respiratory disease and gastroenteritis. On the other hand, the animal experiments suggested that the major viral load following SAFV intraperitoneal inoculation into mice is the pancreas. However, up to date, there have been no reports of SAFV being detected from patients with pancreatitis. This report presents a unique case which developed a relapsing acute pancreatitis (AP) after hand, foot and mouth disease and suspected of SAFV-1 infection. A 2-year-old boy was admitted to the hospital because of severe abdominal pain. His serum amylase and lipase levels were elevated. Enhanced computed tomography showed pancreatic swelling and dilation of main pancreatic duct, leading to the diagnosis of severe AP. The viral genome of SAFV-1 was detected by RT-PCR from fecal samples. Furthermore, serum neutralization titer against SAFV was elevated during the AP and decreased after 1 year. These findings strongly suggest the patient developed an SAFV-1 infection concurrent with AP. Therefore, we propose a cohort study is required to clarify a relation between SAFV and the AP hereafter.
Acute pancreatitis (AP) in children occurs from many causes, such as drugs, infection, trauma, and anatomic anomalies of the pancreaticobiliary junction. Suzuki et al. (1) reported that among 145 AP patients, 79 (54.5%) children had congenital anomalies of the pancreaticobiliary system, 19 (13.1%) had drug-induced AP, 14 (9.7%) had trauma-induced AP, 13 (9.0%) had multisystem disease, and five (3.4%) had infection (3.4%). As the infectious agents associated with AP, several viruses (mumps, coxsackie, hepatitis B, cytomegalovirus, varicella-zoster virus, herpes simplex virus) were reported (2). On the other hand, Saffold cardiovirus (SAFV) was recovered from a stool sample in 2007 (3). Several reports have proposed the possibility that SAFV is associated with respiratory disease and gastroenteritis (4-6). However, there have been no reports of SAFV being detected from patients with pancreatitis. In this report, we present a unique case suggesting that a relapsing AP after hand, foot and mouth disease is associated with SAFV-1 infection. The informed consent has been obtained from the parents of the patient.

A 2-year-old boy with a past history of Kawasaki disease at the age of one was observed closely without therapy with a diagnosis of hand, foot and mouth disease on July 15 2012 in Kyoto. After 4 days, he was admitted to the hospital because of a sudden onset of severe central abdominal pain and vomiting on July 19. He had not
been exposed to any new medication. In addition, he had no apparent abdominal bruising, meningeal signs, or evidence of lower respiratory disease. His serum amylase and lipase levels were elevated (amylase: 907IU/L, pancreatic amylase: 880IU/L, elastase I: 1,700IU/L, lipase: 1570U/L), whereas the results of other blood and urine findings were normal (Table 1). Enhanced computed tomography (CT) showed pancreatic swelling and dilation of the main pancreatic duct and the inflammation had extended to the lowest part of left kidney, leading to the diagnosis of severe AP; pseudocysts and gallstones were not evident (figure 1). A follow-up magnetic resonance cholangiopancreatography (MRCP) did not show any anatomical abnormalities, such as an anomalous arrangement of the pancreaticobiliary ducts (data not shown). Therefore, we suspected an AP caused by viral infection and tried the virus isolation. Nasopharyngeal swabs and fecal samples were collected at the 2nd hospital day. Sera were collected at the 20th hospital day and at 6 months after discharge. To isolate various viruses, we used 6 different cell lines (HeLa, RD-18S, Hep-2, A549, MDCK, Vero E6). These cells are sensitive to the viruses which are known to be the causative agents for pancreatitis: coxsackievirus (A9, A16, B), echovirus, respiratory syncytial virus, adenovirus, influenza virus, human metapneumovirus, severe acute respiratory syndrome virus, and mumps virus. However, these pathogens were not isolated from his nasopharyngeal swabs and fecal samples. And we also used an
enzyme-linked immunosorbent assay (Rotaclone®; Premier Bioscience Cincinnati, Ohio, USA) to check rotavirus in his fecal sample, but the result was negative. Serum antibody titers to mumps virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, and herpes simplex virus were not elevated. SPINKS1, PRSS1, and CFTR mutations were not detected, nor did the patient show any signs of autoimmune disease. Furthermore, his serum levels of triglyceride, IgG, and IgG4 were within normal range (table 1). The patient was maintained on total parenteral nutrition and received protein synthesis inhibitor (ulinastatin), and anti-acid (famotidine) therapy. During his 5-month-hospitalization, the AP relapsed three times. Since the patient was discharged, he has been under a follow-up care as an outpatient and has no episode of AP relapse in the 24 months from the last episode.

In the present paper, we focused on SAFV since picornaviruses are reported to cause pancreatitis (2, 7, 8) and the animal experiments showed that the major viral load following SAFV intraperitoneal inoculation into mice is the pancreas (9). We attempted to detect SAFV from the stored stool specimen using a nested reverse transcription polymerase chain reaction (RT-PCR). The extraction of RNA sample was performed by using the QIAamp Viral RNA Mini Kit (QIAGEN in accordance with the method of Jan Felix Drexler et al (10)). The bands of the 5’-noncoding region were clearly detected in first round PCR (1,184 bp) using SuperScript III One-Step RT-PCR
System with Platinum Taq (Invitrogen, Carlsbad, CA, USA) and nested PCR (947 bp) using Platinum Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA). The virus was identified as SAFV-1 (GenBank accession no. AB769410) by the direct sequence analysis (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, Massachusetts, USA) of the VP1 region and basic local alignment search tool (BLAST). Furthermore, we tested the serum neutralization titer against SAFV-3 (cDNA-derived JPN08-404 (11) was used as an antigen) on HeLa-N cells (which are highly susceptible to SAFV-3) (12) using the stored sera at the 20th hospital day and at 6 months after discharge. The serum titer against SAFV-3 was elevated (×192) during the patient’s AP and the titer decreased (×10). Although there were differences between values of neutralizing activity for SAFV-2 and SAFV-3, cross-reactivity has been reported (13); thus, the neutralizing activity to SAFV-1 cross-reactive with SAFV-3 is contained in the patient’s sera with low level. The above data strongly suggest that there was SAFV infection during the AP. In addition, negative sera findings suggest that the AP is not caused by hereditary pancreatitis, autoimmune pancreatitis, or metabolic disease such as hypertriglyceridemia. Although the investigation of pancreatic structure by the aid of CT and MRCP has not been performed, re-examination by the endoscopic retrograde cholangiopancreatography (ERCP) should be performed after the affected child is grown. The affected pancreatic
tissue has not been investigated, either. Therefore, it may be insufficient to emphasize the direct relationship between SAFV infection and AP at present.

Itagaki et al. (4) reported SAFV-2 is a cause of upper respiratory tract illness that exhibits a pathogenicity similar to that of Coxsackievirus B. And it was also found in the stool of children with nonpolio acute flaccid paralysis (14). There are many reports about the relationship of SAFV to disease in human, but it still remains unclear. Craighead et al. (15) reported encephalomyocarditis virus which has a high homology with SAFV appears to cause diabetes mellitus by reducing the mass of functional beta cells of the islets of Langerhans. Sorgeloos et al. (9) presented that the major viral load following SAFV intraperitoneal inoculation into 129/Sv mice is the pancreas. On the other hand, Tapia et al. (16) recently reported that SAFV had no statistically significant association with islet autoimmunity. Further studies are required to determine its precise role as a human pathogen although the present case suggested the possibility of the relation between SAFV and AP.

In conclusion, the isolation of various viruses on several cells by using nasal smear and stool specimens obtained at the time of hospitalization was negative as described above. However, SAFV-1 was detected in stool specimens by RT-PCR. Furthermore, the antibody titer against SAFV in the serum collected at the time of first relapse during hospitalization was significantly elevated. In addition, we experienced
another case with AP, in which SAFV was detected in the stool specimen (data not shown). These results strongly suggest there was the SAFV infection in the patient during the AP. However, in this case, the possibility of coxsackievirus B or other enteroviruses cannot be excluded although the isolation of those viruses was negative. The accumulation of cases will be indispensable to make the relation between the SAFV infection and the pancreatitis clearer.

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Conflict of interest

None to declare.
References


Figure Legends

Figure 1; Enhanced computed tomography

Enhanced computed tomography shows the pancreatic swelling and dilation of the main pancreatic duct and the inflammation is extended to the lowest part of the left kidney, leading to the diagnosis of severe AP grade II: pseudocysts and gallstones are not evident.
Table 1: Laboratory data on admission.

<table>
<thead>
<tr>
<th>Peripheral blood</th>
<th>Biochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count</td>
<td>Sodium</td>
</tr>
<tr>
<td>11,210 /μl</td>
<td>137 mEq/l</td>
</tr>
<tr>
<td>Neutrophilic granulocyte</td>
<td>Potassium</td>
</tr>
<tr>
<td>86.2 %</td>
<td>4.3 mEq/l</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>Chloride</td>
</tr>
<tr>
<td>10.8 %</td>
<td>107 mEq/l</td>
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<tr>
<td>Hemoglobin</td>
<td>Calcium</td>
</tr>
<tr>
<td>11.3 g/dl</td>
<td>9.6 mg/dl</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>Aspartate</td>
</tr>
<tr>
<td>32.5 %</td>
<td>32 U/l</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Alanine</td>
</tr>
<tr>
<td>38.1 10⁴ /μl</td>
<td>23 U/l</td>
</tr>
<tr>
<td></td>
<td>Lactic dehydrogenase</td>
</tr>
<tr>
<td></td>
<td>239 U/l</td>
</tr>
<tr>
<td>Serological test</td>
<td>Total bilirubin</td>
</tr>
<tr>
<td>IgG</td>
<td>692 mg/dl</td>
</tr>
<tr>
<td>IgG4</td>
<td>Urea nitrogen</td>
</tr>
<tr>
<td>16.6 mg/dl</td>
<td>15.1 mg/dl</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>Creatinine</td>
</tr>
<tr>
<td>3 U/ml</td>
<td>0.21 mg/dl</td>
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<tr>
<td>Antinuclear antibodies</td>
<td>Uric acid</td>
</tr>
<tr>
<td>&lt;×40</td>
<td>4.8 mg/dl</td>
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<tr>
<td>C-reactive protein</td>
<td>Total protein</td>
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<tr>
<td>0.7 mg/dl</td>
<td>6.8 g/dl</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td></td>
<td>4.1 g/dl</td>
</tr>
<tr>
<td></td>
<td>Creatine phosphokinase</td>
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<tr>
<td></td>
<td>51 U/l</td>
</tr>
<tr>
<td>Antibody</td>
<td>Total cholesterol value</td>
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<tr>
<td>mumps virus</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>negative</td>
<td>86 mg/dl</td>
</tr>
<tr>
<td>hepatitis B virus</td>
<td>Amylase</td>
</tr>
<tr>
<td>negative</td>
<td>907 U/l</td>
</tr>
<tr>
<td>hepatitis C virus</td>
<td>Pancreatic amylase</td>
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<tr>
<td>negative</td>
<td>880 U/l</td>
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<tr>
<td>Cytomegalovirus</td>
<td>Elastase I</td>
</tr>
<tr>
<td>negative</td>
<td>1,700 U/l</td>
</tr>
<tr>
<td>herpes simplex virus</td>
<td>Lipase</td>
</tr>
<tr>
<td>negative</td>
<td>1,570 U/L</td>
</tr>
</tbody>
</table>

The sub-item with abnormally high level is underlined.