Reactivation with Pancreatin of Coproantibody-Neutralized Virus in Ground Beef

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ABSTRACT

Viruses transmissible through food are sometimes shed in association with coproantibody, which may preclude their detection by conventional means. We had shown previously that a large volume of food extract can be tested in cell culture, and that the inoculum can be transferred from one type of cell culture to another to afford a broader spectrum of virus sensitivity. Here we report that pancreatin is capable of reactivating the majority of coproantibody-neutralized poliovirus and that pancreatin-treated extract from ground beef samples can be tested in large volumes and transferred among cell cultures as described above.

The problem of virus transmission through foods is gaining worldwide recognition (2). Most foodborne viruses are shed in association with coproantibody from humans emanate from the human intestines (2). Late in the course of infection, these viruses may be shed with coproantibody attached to them. Coproantibody-neutralized virus is noninfectious in the cell cultures in which detection may be attempted, but can be reactivated in the digestive tract (5). Several methods have been developed for the isolation of viruses from a wide variety of foods, including ground beef (6). Though detection procedures that involve treatment with trichlorotrifluoroethane (TTF; Freon TF, DuPont, Wilmington, DE) reactivate such viruses and enable them to be detected in cell cultures (7, 8), most other methods do not.

Pancreatin treatment affords an alternate means of reactivating coproantibody-neutralized virus. Enteroviruses are unlikely to be inactivated by proteolytic enzymes (3), though trypsin (present in pancreatin) subtly modifies the coat protein of poliovirus without affecting infectivity (8). Pancreatin should also be capable of activating reoviruses and rotaviruses, which are potentially foodborne, and of freeing viruses that are shed from the intestines in membrane-bound packets (18), so as to improve their detectability in cell cultures. Here we describe a convenient method for pancreatin treatment of food suspensions and extracts to enhance the probability of detecting viruses present. As with other methods we have reported (12, 13), this one permits testing a large volume of food extract per cell culture, if desired, rather than concentrating the extract before testing.

Although cell cultures afford an extremely sensitive substrate, no single cell type is susceptible to all of the foodborne viruses one might hope to detect. We have shown that transferring a food extract inoculum from one type of cell culture to another offers a sensitive detection procedure for a broader range of viruses (13). In the present study, large volumes of pancreatin-treated food extracts were transferred from one cell type to another in order to determine whether these procedures are compatible.

MATERIALS AND METHODS

Poliovirus type 1 (PO1), strain CHAT, was obtained from the American Type Culture Collection, Rockville, MD, and porcine enterovirus type 3 (PE3) was obtained from E. H. Bohl, Ohio Agricultural Research and Development Station, Wooster, OH. Coproantibody-neutralized poliovirus type 1 was prepared as previously described (4, 16), using coproantibody from feces obtained from the State Laboratory of Hygiene, Madison, WI; neutralization was shown to exceed 99.99%. Buffalpo green monkey (BGM) kidney cell cultures were originally obtained from D. R. Dahling, U.S. Environmental Protection Agency, Cincinnati, OH, and swine testicle tissue cultures were originally obtained from the American Type Culture Collection, Rockville, MD. Both types of cultures were maintained in our laboratory as previously described (4).

In earlier experiments, a pancreatin stock solution was made by dissolving one Oxoid pancreatin tablet (Difco, Detroit, MI) in 100 ml of deionized water. Later, a "10 x" pancreatin solution (Grand Island Biological Company, Grand Island, NY) was used the same as the original "Oxoid" stock solution. Representatives of both suppliers agreed that pancreatin concentrations were the same (personal communications). Preliminary experiments showed that cell cultures would tolerate a maximum of 2 ml of pancreatin stock solution per 25 ml of culture inoculum, provided the inoculum was removed within 16 to 24
The inoculum by 5 ml of maintenance medium, and continued approximately 9.5 ml concentrated Eagle’s medium with calf rocking for 1 h at 37°C, followed by the addition of 5 ml of maintenance medium containing agar and further incubation at 37°C during examination for viral cytopathic effects of three stages in the procedure: “Option 1” — after addition of the buffer to the ground beef but before addition of the Cat-Floc (2 ml pancreatin/100 ml buffer), “Option 2” — after filtration but before inoculation into cell culture (2 ml pancreatin/90-100 ml extract) and “Option 3” — after incubation of the inoculated cell cultures overnight (0.4 ml pancreatin/5 ml maintenance medium). Because enteroviruses receptors on host cells are known to be susceptible to proteases (11), it was important to show that the host cells’ sensitivity to virus was not affected by pancreatin treatment under these conditions. Cultures were incubated for 12 h at 37°C with 5 ml of maintenance medium to which 0.4 ml of pancreatin stock solution had been added. These and control cultures that had not been treated with pancreatin gave essentially identical titers when used to plaque PO1 (1.4 × 10^3 and 1.5 × 10^3 PFU/ml, respectively), whereas no plaques formed in cultures of each set after inoculation with PO1-cAb. Option 3 was tested only once: it did not reactivate coproantibody-neutralized virus, and cytopathic effects occurred in the cell cultures due to prolonged incubation (24 to 30 h) with the pancreatin present. In both Options 1 and 2, the pancreatin was reacted with the sample for 30 min before the next step in the procedure. Pancreatin was tested with PO1 neutralized by coproantibody (PO1-cAb) and, to detect any adverse effects of the treatment, with free PO1 and PE3.

**RESULTS**

The first set of experiments compared the addition of pancreatin either to the buffer at the start of the procedure (Option 1) or to the sample extract after filtration (Option 2). Both a “high” inoculum (1.0 × 10^4 PFU/20 g beef sample) and a “low” inoculum (1.0 PFU/20 g beef sample) of PO1 were used. No deleterious effects on viral recovery were seen; neither was there a difference based on time of pancreatin addition (Tables 1 and 2). The most probable number of cytopathic units (MPNCU) recovered per sample was estimated by a method based on that described by Sobsey (15). MPNCU titers of enteroviruses have been found to be equivalent to PFU, but the plaque assay is more precise because it is usually based on a larger number of observed events (1,13). In the low-inoculum trials, both Option 1 and Option 2 showed four of eight samples positive for virus; the results did not differ significantly from what would be expected for 100% recovery of inoculated virus, by the Poisson distribution (7).

The above experiments were repeated using PO1-cAb virus. Levels of virus inoculated were estimated on the basis of the number of PFU in the suspension before neutralization by coproantibody, times the applicable dilution factor. Recoveries of virus by Options 1 and 2 were similar at high levels of inoculation (Table 1) and probably did not differ significantly at low levels of inoculation (Table 2). Both TTF control samples had three of eight samples positive (Table 2), samples processed by Option 1 had two of eight samples positive, and samples processed by Option 2 had four of eight samples.
TABLE 1. Recoveries of virus from 20-g samples of ground beef inoculated with at least 10⁴ PFU in preliminary experiments.

<table>
<thead>
<tr>
<th>Virusb</th>
<th>Inoculum (PFU/sample)</th>
<th>Reactivation treatmentc</th>
<th>Virus recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO1</td>
<td>1.0 × 10⁴</td>
<td>Option 1</td>
<td>9.5 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>1.0 × 10⁴</td>
<td>Option 2</td>
<td>1.0 × 10⁴</td>
</tr>
<tr>
<td>PO1-cAb</td>
<td>1.2 × 10⁴</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.2 × 10⁴</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2.6 × 10⁴</td>
<td>Option 1</td>
<td>1.9 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>2.6 × 10⁴</td>
<td>Option 2</td>
<td>2.0 × 10⁴</td>
</tr>
</tbody>
</table>

*Assays were done by the "high" virus level procedure (Fig. 1); for these preliminary experiments, each line represents a single sample tested.

b PO1, poliovirus 1; PO1-cAb, poliovirus 1 neutralized by coproantibody.

PO1, poliovirus 1; PO1-cAb, poliovirus 1 neutralized by coproantibody.

Option 1, additional of pancreatin to sample suspension before Cat-Floc (Fig. 1); Option 2, pancreatin treatment of the filtered sample extract; None, extraction as in Fig. 1, but with no addition of pancreatin.

PFU recovered per sample/PFU inoculated per sample X 100.

TABLE 2. Recoveries of virus from sets of eight 20-g samples of ground beef after low level inoculation using only homologous cell cultures.

<table>
<thead>
<tr>
<th>Virusb</th>
<th>Inoculum (PFU/sample)</th>
<th>Reactivation treatmentc</th>
<th>Cultures with CPEd</th>
<th>Virus recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO1</td>
<td>1.0</td>
<td>Option 1</td>
<td>0 2 2 4</td>
<td>0.86 86</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>Option 2</td>
<td>0 3 1 4</td>
<td>1.03 103</td>
</tr>
<tr>
<td>PO1-cAb</td>
<td>1.5</td>
<td>Option 1</td>
<td>2 0 0 6</td>
<td>0.86 57</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>Option 2</td>
<td>3 1 0 4</td>
<td>1.83 122</td>
</tr>
<tr>
<td></td>
<td>TTF</td>
<td>2 1 0 5</td>
<td>1.21 81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTF</td>
<td>3 0 0 5</td>
<td>1.41 94</td>
<td></td>
</tr>
</tbody>
</table>

*Sample extracts + CES were tested directly in homologous cell cultures.

b PO1, poliovirus 1; PO1-cAb, poliovirus 1 neutralized by coproantibody.

Option 1, addition of pancreatin to sample suspension before Cat-Floc (Fig. 1); Option 2, pancreatin treatment of the filtered sample extract; TTF, homogenization of the sample extract with trichlorotrifluoroethane.

e Eight sample extracts per experiment were inoculated into three homologous cell cultures each; reported are the number of sample extracts that caused CPE in 3, 2, 1 and 0 of the inoculated cultures.

f The most probable number of cytopathic units (MPNCU) recovered per sample was estimated from the CPE results by a method based on that of Sobsey (15).

MPNCU recovered per sample/PFU inoculated per sample × 100.

positive. All of these results are consistent with the Poisson distribution. As no significant differences had been seen between pancreatin added to the buffer (Option 1) or pancreatin added to the extract (Option 2), pancreatin was added to the extract in all subsequent experiments.

All remaining experiments entailed inoculation of low levels of virus into each of eight ground beef samples. Each extract was treated to reactivate neutralized virus, whether or not coproantibody had been used, and inoculated first into heterologous cells and then transferred to homologous cells on the second day. It appears that roughly half of the poliovirus and essentially all of the PE3 were recovered by the procedure described (Table 3). Obviously, substantial reactivation of the coproantibody-neutralized virus was achieved by the pancreatin treatment, but some loss of poliovirus may have occurred during incubation in the heterologous (ST) cell cultures.

DISCUSSION AND CONCLUSIONS

Pancreatin has been used previously in overlay media for the enhancement of reovirus titers, though at a lower level than reported in this paper (17); the pancreatin removes the outer reovirus coat. The mechanism for pancreatin reactivation of coproantibody-neutralized virus, though unknown, may be analogous to the reovirus enhancement, with the coproantibody being physically removed from the viral surface. Pancreatin treatment in extracts of ground beef will reactivate coproantibody-neutralized virus at least as well as the TTF extraction of Herrmann and Cliver (10). Furthermore, the addition of pancreatin does not cause problems with the isolation of non-neutralized virus that may be present. However, care must be taken to not leave pancreatin-containing extracts in contact with cell cultures for more than 24 h.

The importance of transferring the food extract inoculum from one type of cell culture to another is self-evident if the quantity of available food sample is limited. Given the possible loss of virus seen in Table 3, one might rather not do a transfer if plenty of sample was available. The relative cost of processing a duplicate set of food samples for testing in a second type of cell culture may be greater, however. Since we reported testing food extracts sequentially in different types of cell cultures (13), a similar procedure has been recommended for virologic testing of shellfish extracts (14).

The procedures outlined in this paper are easy and inexpensive. As food contaminated with fecal matter might...
Option 2, pancreatin treatment of the filtered sample extract (Fig. 1); TTF, homogenization of the sample suspension with trichlorotrifluoroethane. no CPE was seen in any of the heterologous cultures.

TABLE 3. Recoveries of viruses from sets of eight 20-g samples of ground beef after low level inoculation, with first “testing” in heterologous cell cultures.

<table>
<thead>
<tr>
<th>Virus</th>
<th>PFU/sample</th>
<th>Reactivation treatment</th>
<th>Cultures with CPE</th>
<th>Virus recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 2 1 0</td>
<td>MPNCU/sample  %</td>
</tr>
<tr>
<td>PO1</td>
<td>1.0</td>
<td>Option 2</td>
<td>0 0</td>
<td>0.27 27</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>Option 2</td>
<td>1 0</td>
<td>0.70 70</td>
</tr>
<tr>
<td>PO1-cAb</td>
<td>1.5</td>
<td>Option 2</td>
<td>0 2</td>
<td>0.70 47</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>TTF</td>
<td>2 1</td>
<td>0.70 47</td>
</tr>
<tr>
<td>PE3</td>
<td>2.0</td>
<td>Option 2</td>
<td>0 3</td>
<td>2.34 117</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>Option 2</td>
<td>2 0</td>
<td>0.87 109</td>
</tr>
</tbody>
</table>

a Sample extracts + CES were incubated for at least 16 h in heterologous cell cultures before being transferred to homologous cells; no CPE was seen in any of the heterologous cultures.

b PO1, poliovirus 1; PO1-cAb, poliovirus 1 neutralized by coproantibody; PE3, porcine enterovirus 3.

c Option 2, pancreatin treatment of the filtered sample extract (Fig. 1); TTF, homogenization of the sample suspension with trichlorotrifluoroethane.

d Eight sample extracts per experiment were finally inoculated into three homologous cell cultures each; reported are the numbers of sample extracts that caused CPE in 3, 2, 1 and 0 of the inoculated cultures.

The most probable number of cytopathic units (MPNCU) recovered per sample was estimated from the CPE results by a method based on that of Sobsey (15).

1 MPNCU recovered per sample/PFU inoculated per sample × 100.

well contain coproantibody-neutralized virus (2), the ability to detect such neutralized virus could be used as an indication of fecal contamination. The addition of pancreatin to a food extract may also aid in the recovery of low levels of reovirus or rotavirus. When this method is applied to field samples, negative CPE results may be confirmed by “blind” passages in the same type of cell culture and positive results must be validated by further cell culture passage and identification of the cytopathic agent (6). Pancreatin treatment would appear to be appropriate when testing samples of food, and perhaps also water, that are suspected of being contaminated with human enteric viruses.

ACKNOWLEDGMENTS

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REFERENCES