Reovirus Type 1 Is Secreted into the Bile

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Reovirus type 1, known to be a cause of systemic and intestinal disease in mice, is secreted into the bile of adult A/J mice after viremia. Virus found in the bile in concentrations higher than those in blood may indicate that reovirus type 1 is actively transported into the bile. The transport of virus was independent of levels of virus-specific immunoglobulin A antibody. Modifications of the virus that occurred during transport did not discernibly affect the infectivity of the virus. Entry of virus into the bile may be an important mechanism by which an enteric virus that produces systemic disease reenters the intestine for transmission.

Several viruses that cause systemic disease enter the host via the gastrointestinal tract. These include the picornaviruses, such as poliovirus and hepatitis A virus, coronaviruses, and the reoviruses. Although these viruses have specific target organs, such as the liver for hepatitis A virus, they are also recoverable from feces, and it is their presence in feces that accounts for the transmission of these agents. To determine how virus is shed into the intestinal lumen during the systemic phase of illness, we studied reovirus infection in the mouse.

Our studies with reovirus type 1 strain Lang (reovirus type 1/L) demonstrate that after peroral inoculation of adult A/J mice, the only cells infected in the intestine are the epithelial cells in the ileum (16, 19, 20). However, 24 h after intravenous (i.v.) inoculation, the highest titer of virus was present in the lumen of the duodenum (3.45 ± 0.49 [standard error] log₁₀ PFU) (15). This finding suggests that reovirus type 1 enters the lumen of the upper bowel by a mechanism other than infection of epithelial cells. We hypothesized that the virus might enter the lumen by a secretory mechanism and tested this hypothesis by collecting and determining the titer of bile from adult A/J mice after i.v. inoculation with reovirus type 1/L.

We found that reovirus type 1/L was secreted into the bile and reovirus type 3 strain Dearing (3/D) was not. Secretion of virus into the bile appears in part to be dependent upon virus-specific factors.

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MATERIALS AND METHODS

Mice. Adult female A/J mice (Jackson Laboratory, Bar Harbor, Maine), 8 to 12 weeks old, were fed a house diet (Purina, St. Louis, Mo.) ad libitum. There was no evidence by enzyme-linked immunosorbent assay of a systemic humoral immune response to reovirus in any mouse used for these experiments (17).

Virus. Reovirus type 1/L and reovirus type 3/D were previously described (4). For mouse inoculation, a stock of reovirus that was passed twice in L cells was purified by substituting ultrasonic disruption (Ultrasonic 200; Branson Sonic Power Co., Danbury, Conn.) for cell homogenization.

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Reovirus were the volume of enterohepatic circulation transported into the bile in the virus-inoculated tractation of protein. It is first determined that IgA is equal to 3/D in the systemic liver cells (13). It was not recovered from bile in the ileum of the mouse (15). Our findings were consistent with this; i.e., when reovirus type 3/D was given i.v., no infectious virus was found in the secretions in any bowel segment. If a secretory mechanism is involved in the entry of virus into the lumen of the intestine during viremia, we would predict that reovirus type 3/D would not be present in the bile. This was found to be the case. In mice inoculated i.v. with reovirus type 3/D, no infectious virus was found in the ileum after the first 6 h after virus inoculation. Additionally, when tritiated uridine was used to label the reovirus type 3/D genome, there was no evidence of reovirus particles in the bile after i.v. inoculation. Finally, to determine whether the absence of infectious virus in the bile was due to a direct effect of bile on reovirus infectivity, purified reovirus types 1/L and 3/D were incubated with bile for 1 h at 37°C. There was no decrease in infectious virus after bile incubation compared with untreated controls. Thus, reovirus type 3/D is not recoverable from bile in an infectious or noninfectious form.

**RESULTS**

To determine whether reovirus was present in the bile immediately after the initiation of viremia, three to five mice were inoculated i.v. with 10^6 PFU after cannulation of the gallbladder. Reovirus type 1/L appeared in the bile at 0.5 h after inoculation, and the titer of virus reached a steady state within 2 to 3 h after inoculation. The concentration of reovirus type 1/L recovered from the blood was approximately 1 log less than its concentration in the bile at similar time points (Fig. 1). Furthermore, the presence of the virus in the bile remained at a steady state throughout the viremia (Fig. 1). These findings suggest that the virus is actively transported into the bile against a concentration gradient.

Since reovirus type 1/L specifically infects the ileum, we next determined whether reovirus type 1/L had an enterohepatic circulation by inoculating mice intraluminally directly into a duodenal loop with 10^6 PFU of reovirus type 1/L. We were not able to recover virus in the bile during the first 12 h after infection. However, from 12 to 24 h, two of eight mice had evidence of virus in the bile. Thus, low levels of virus were found in some mice during the first 24 h after gut administration of virus.

Several studies found that dimeric immunoglobulin A (IgA) is associated with the clearance of antigens from systemic circulation by cotransport through the liver (2, 11), and it was hypothesized that for antigens that enter systemic circulation, antigen-antibody complexes are important in antigen removal. To determine whether the mechanism of virus secretion into the bile involves cotransport of virus in immune complexes, we used a standard solid-phase enzyme-linked immunosorbent assay for the detection of antireovirus antibodies (17). No virus-specific IgA was detected in the bile, indicating that antibodies are not critically involved in this system.

Modification of the antigen may occur during processing within the liver cell before the secretion of virus into the bile (13). Previous studies with several different viruses suggested that high levels of virus are required for uptake by liver cells with subsequent infection of the hepatocyte, further suggesting that each virus is either sequestered or modified before entry into the hepatocyte (7,8). It is, therefore, possible that by measuring only infectious particles, only a small fraction of the viral antigen was being assayed in the bile. To evaluate whether reovirus type 1/L may be modified to a noninfectious form, we compared the concentration of infectious virus with the total concentration of virus particles by using virus radiolabeled with [3H]uridine. The presence of radiolabeled virus in the bile reached a steady state at approximately 2 h after infection, and the calculated particle-to-PFU ratio was similar to that of the input inoculum (data not shown).

Bile accounts for only a fraction of the total volume of fluid secreted into the lumen of the intestine. The remainder of intestinal luminal fluid is derived from sources such as saliva, gastric juice, pancreatic juice, and crypt cells. To examine whether biliary secretion accounted for the entire amount of infectious reovirus present in the intestinal lumen after i.v. inoculation, the contents of the intestinal lumen were filtered for infectious virus after the bile was collected (Table 1). After common bile duct ligation, reovirus type 1/L was present within the lumen in all segments of the bowel and was distributed to segments of the intestine as previously reported at 24 h after infection (15).

To determine whether the entry of reovirus type 1/L into the bile was a property shared by other reovirus types, we tested reovirus type 3/D. Previous studies with reovirus type 3/D showed that it does not infect the ileum of the mouse (15). Our findings were consistent with this; i.e., when reovirus type 3/D was given i.v., no infectious virus was found in the secretion in any bowel segment. If a secretory mechanism is involved in the entry of virus into the lumen of the intestine during viremia, we would predict that reovirus type 3/D would not be present in the bile. This was found to be the case. In mice inoculated i.v. with reovirus type 3/D, no infectious virus was found in the ileum during the first 6 h after virus inoculation. Additionally, when tritiated uridine was used to label the reovirus type 3/D genome, there was no evidence of reovirus particles in the bile after i.v. inoculation. Finally, to determine whether the absence of infectious virus in the bile was due to a direct effect of bile on reovirus infectivity, purified reovirus types 1/L and 3/D were incubated with bile for 1 h at 37°C. There was no decrease in infectious virus after bile incubation compared with untreated controls. Thus, reovirus type 3/D is not recoverable from bile in an infectious or noninfectious form.

**DISCUSSION**

We examined whether reovirus type 1/L entered the gastrointestinal lumen by a secretory mechanism. It was found that infectious reovirus type 1/L appeared in the bile of mice inoculated i.v. with 10^6 PFU of reovirus type 1/L. Titers of virus from the lumen of the bowel or segments of intestine were determined after the termination of 4 h of bile collection. Titers are expressed as the geometric mean (log_{10}) of three mice ± the standard error of the mean.

**TABLE 1.** Reovirus type 1/L titer in the intestine

<table>
<thead>
<tr>
<th>Bowel segment</th>
<th>Lumen (PFU/ml of fluid) ± SEM</th>
<th>Segment (PFU/g of protein) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>4.00 ± 0.44</td>
<td>7.54 ± 0.45</td>
</tr>
<tr>
<td>Jejunum</td>
<td>3.21 ± 1.15</td>
<td>7.10 ± 0.42</td>
</tr>
<tr>
<td>Ileum</td>
<td>2.31 ± 1.27</td>
<td>7.57 ± 0.41</td>
</tr>
<tr>
<td>Colon</td>
<td>3.01 ± 0.11</td>
<td>6.88 ± 0.12</td>
</tr>
</tbody>
</table>

* Mice were inoculated i.v. with 10^6 PFU of reovirus type 1/L. Titers of virus from the lumen of the bowel or segments of intestine were determined after the termination of 4 h of bile collection. Titers are expressed as the geometric mean (log_{10}) of three mice ± the standard error of the mean.
during the first hour after viremia was established. Since the replicative cycle for reovirus is greater than 18 h, we believe that the virus recovered from the bile was from the input inoculum. Moreover, radiolabeled virus was recovered in quantities that indicate that the input inoculum did transit into the bile. In addition, virus appeared in the bile at 10-fold-higher titers than in the blood, suggesting that the virus was actively transported into the biliary system. Thus, the liver, which is the major site for detoxification and elimination of foreign substances, including drugs and particulate antigen, also is capable of transporting infectious virus.

Since reovirus type 1 infects the ileum and is secreted into the bile, an enterohepatic circulation was hypothesized. However, we were not able to recover virus in the bile during the first 12 h after peroral infection. Since these results may reflect replication of the virus in hepatocytes or biliary duct epithelium, with subsequent secretion of the virus into bile, enterohepatic passage of virus is unproven. The presence of reovirus in the bile does not account for the total content of infectious virus present in the intestinal lumen at 4 h after infection. Thus, other sites of elaboration of intestinal fluids may also serve as sites for the secretion of infectious virus. Whether a common pathway is shared by these additional sites is currently under investigation.

Reovirus types 1/L and 3/D do not share the same properties in relation to secretion in the bile or in the intestinal lumen. We found that reovirus type 3/D did not maintain viremia (data not shown) and that the failure to induce sustained viremia directly correlated with our inability to recover infectious virus from the bile. These results suggest either that entry of the virus into bile requires a persistent viremia or that the hepatocytes do not necessarily participate in the elimination of reovirus type 3 from the host (clear the viremia). Alternatively, it is known that reovirus type 3 infects the biliary duct epithelium in the neonatal or suckling mouse (9, 10). It is probable that reovirus type 3 binds to the duct epithelium through virus-specific receptors (3) that are not shared by reovirus type 1. The mechanisms of secretion of the virus may therefore be shared by types of reovirus, but the ability to recover virus in an infectious form may differ owing to virus tropism to liver cell-binding sites. Further work is needed to establish the mechanism of elimination of reovirus type 3.

Although much information now exists on the transport mechanisms of selected substances, such as dimeric IgA, horseradish peroxidase, and certain bile acids, our understanding of hepatocellular transport is incomplete (1, 14). Our studies indicate that pathogenic organisms can enter the bile directly, as opposed to entering the lumen by infection of either hepatocytes or biliary lining cells or as complexes associated with dimeric IgA. Thus, the development of a full understanding of the consequences of the transport of virus in secretions such as bile may directly influence our capacity to control the spread of viruses that cause systemic infection.

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LITERATURE CITED