Pathophysiological aspects of coxsackievirus B intestinal infection¹⁻³

Roger M. Loria, Ph.D., Sidney Kibrick, M.D., Ph.D., and Selwyn A. Broitman, Ph.D.

Relatively little is known about the pathophysiological events that are associated with viral enteric infections. The susceptibility of the adult mouse to infection with group B coxsackievirus and the absence of clinical manifestations in this host indicated that this would make a useful model for studying such events. The marked similarities between infection with this agent in the mouse and man, moreover, suggested that the results of such studies might also have application to man. Accordingly, mice infected perorally (po) or intraperitoneally (ip) with a group B coxsackievirus were examined for the effects of such infection on glucose and leucine absorption from the gut. In addition, the effects on epithelial cell division and migration rate in the gut were determined.

Materials and methods

The materials and procedures used in these experiments have previously been described (1, 2). A pool of coxsackievirus B5, prepared in HeLa cell cultures was used as the infecting viral agent. All studies were done with young adult (33 days old) CD-1 male mice. A minimum of 32 samples were studied for each parameter examined; generally, however, larger numbers were used. Intestinal tissues were prepared for histological examination by the procedure of Loria et al. (3). Intestinal absorption in mice were measured by a double-label radioisotope perfusion technique developed in these laboratories (4). For the absorption experiments, mice were anesthetized with sodium pentobarbital. Through a midline abdominal incision, a polyethylene tube was inserted and tied securely into the small intestine just below the ligated pylorus; a second tube was inserted just above the cecum. A sigmamotor pump was calibrated to deliver 0.18 ml of perfusate per minute. After 30 min of equilibration, intestinal effluent samples were taken at 10 min intervals for 1 hr. Concentrations of substrate and polyethylene glycol were determined by scintillation counting in a Beckman LS-200B counter.

The procedure of Kopriva and LeBlonde (5) for autoradiography was used for studies on small intestine epithelial cell migration (6).

Results

Infection with coxsackievirus B5 in the mouse; histological observations on the gut

Mice were infected po with 1 x 10⁶ pfu coxsackievirus B5 per animal. Histological examination of the gut at 2, 24, and 72 hr after infection was not remarkable. No evidence of an inflammatory response was observed.

To verify this finding, mice were infected perorally with a larger virus dose (5 x 10⁶ pfu/animal). Virus titers per gram of washed intestinal tissue at 2, 24, and 72 hr postinfection were 10⁷, 10⁴, and 4 x 10¹ pfu, respectively. Despite virus titers as high as 10⁷ pfu/g tissue, no evidence of an inflammatory response or of morphologic changes was found (see Fig. 1). Mice infected with a similar virus dose by the ip route also showed no apparent pathology in the gut despite the presence of virus in high titer in this organ (2).

The demonstration that the mouse develops no pathological changes in the gut after either po or ip infection with group B coxsackievirus indicates that this host-virus system is a suitable model for studying the

¹ From the Departments of Microbiology and Academic Pathology, Virginia Commonwealth University, Richmond, Virginia 23298 and the Departments of Pediatrics and Microbiology, Boston University School of Medicine, Boston, Massachusetts 02118.
² Supported in part by U.S.P.H. NIH Grant HL-18152-02, AM-17202-03, and CA-16750-02.
³ Address reprint requests to: Roger M. Loria, Ph.D., Virginia Commonwealth University, MCV Station, P. O. Box 847, Richmond, Virginia 23298.
⁴ Assistant Professor of Microbiology and Academic Pathology. Recipient of the American Diabetes Association, Inc., Young Investigator Development Award.
⁵ Professor of Pediatrics and Microbiology, Boston University School of Medicine.
⁶ Professor of Microbiology and Nutritional Sciences, Boston University School of Medicine.
Intestinal perfusion experiments in mice infected perorally with coxsackievirus B5

The absorption of specific substrates from the intestinal tract can be estimated by in vivo intestinal perfusion techniques. For example, the difference between concentrations of a substrate in the perfusate and effluent yields the amount of substrate absorbed. During the course of in vivo perfusion, however, water may leave or enter the intestine, producing alterations in substrate concentration. To correct for this effect, polyethylene glycol (PEG), the 4,000 molecular weight polymer, is widely used as a nonabsorbable volume marker. From the ratio \( \frac{\text{PEG}_{\text{effluent}}}{\text{PEG}_{\text{perfusate}}} \), the net water flux can be measured and the appropriate volume correction made. PEG is accepted as the standard marker because of its insignificant absorption in the intestine of man and experimental animal hosts (9-11).

**Glucose absorption.** CD-1 male mice were infected po with \( 10^8 \) pfu of coxsackievirus B5. Three days later, in vivo intestinal perfusion experiments were performed by a double label radioisotope technique (6) using \(^{14}\)C PEG and \(^3\)H-glucose in the perfusate. At this time, virus titers in the enteric tissue had dropped to \( 4 \times 10^4 \) pfu/g. Matched, uninfected mice were also examined as controls. Glucose absorption from the small intestine of non-infected mice was 10.2 mg/hr as compared with 12.9 mg/hr, for the infected mice, a 26.4% increase (see Table 1).

**L-leucine absorption.** These experiments were repeated, substituting tritiated L-leucine for glucose as the substrate in the perfusate solutions. The absorption of L-leucine from the small intestine of non-infected control mice was 2.4 mg/hr while the absorption for the infected mice was 2.8 mg/hr (16.7% more than their matched controls) (see Table 2).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Perfusion studies of glucose absorption in intestine of the mouse following infection with coxsackievirus B5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of infection</td>
<td>Glucose absorbed in mg/hr</td>
</tr>
<tr>
<td>Control group*</td>
<td>10.2</td>
</tr>
<tr>
<td>Peroral</td>
<td>12.9</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Mice were infected 3 days before perfusion with \( 1 \times 10^8 \) pfu virus in a 0.5 ml inoculum. ** Mean values, based on six 10 min samples per mouse and six to eight animals per group. Statistically significant by the paired Student's \( t \) test; \( P < 0.001 \).}

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Perfusion studies of L-leucine absorption in intestine of the mouse following infection with coxsackievirus B5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of infection</td>
<td>Leucine absorbed in mg/hr</td>
</tr>
<tr>
<td>Control group*</td>
<td>2.4</td>
</tr>
<tr>
<td>Peroral</td>
<td>2.8</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Mice were infected 3 days prior to perfusion with \( 1 \times 10^8 \) pfu virus in a 0.5 ml inoculum. ** Mean values, based on six 10 min samples per mouse and six to eight animals per group. Statistically significant by analysis of variants; \( P < 0.05 \).
Water flux. Net water flux was measured from the changes in PEG concentration in the perfusate-effluent and original solution. No significant changes were noted.

**Intestinal perfusion experiments in mice intraperitoneally infected with coxsackievirus B5**

Mice were infected by the ip route with 10⁶ pfu coxsackievirus B5; 3 days later, in vivo intestinal perfusion experiments were done. At this time, virus titers in the enteric tissue were still being maintained at a high level (2 × 10⁷ pfu/g) (2). An increase in glucose and leucine absorption of 17.6 and 25.0% respectively was observed as compared with non-infected controls (see Tables 1 and 2). Since increased absorption was noted following both ip and po infection, the data suggest that there are more than one mechanism which play a role in intestinal absorption after coxsackievirus B infection.

**Intestinal perfusion experiments in mice infected perorally with S. typhimurium**

In contrast to infection with coxsackievirus B5, infection with *S. typhimurium*, a bacterial enteric pathogen, resulted in a significant decrease in absorption of glucose. In these experiments, intestinal perfusion was carried out at 7 to 14 days after infection. Glucose absorption in mice challenged with *S. typhimurium* but that failed to become infected was 8.2% less than for the noninfected controls, while animals challenged and shown to be infected had a decrease in glucose absorption of 36.4% over their noninfected controls (see Table 3).

**TABLE 3**

Perfusion studies of glucose absorption in intestine of mouse following peroral infection with *S. typhimurium*

<table>
<thead>
<tr>
<th></th>
<th>No. of mice</th>
<th>Glucose absorbed in mg/hr</th>
<th>% Decrease in absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>12</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td>Challenged not infected</td>
<td>15</td>
<td>26.7</td>
<td>8.2</td>
</tr>
<tr>
<td>Challenged infected</td>
<td>6</td>
<td>18.5</td>
<td>36.4</td>
</tr>
</tbody>
</table>

* Mice were infected with 10⁶ organisms in a 0.5 ml inoculum 7 to 14 days before perfusion. * Mean values, based on six 10 min samples per mouse.

**Intestinal epithelial cell migration in mice infected perorally with coxsackievirus B5**

Cell division in the small intestine epithelium takes place in the crypt cells. These cells migrate up the lateral edge of the villus to the extrusion zone at the villus tip, where they are sloughed into the lumen. Parenterally administered, tritiated thymidine is taken up by dividing cells only. Therefore, radioactive thymidine is initially found in the crypt cells. The vertical movement of these radioactive-labelled cells can be used as an index of both cell migration rate and cell division rate for the epithelial cells in the small intestine. Using this technique, the intestinal epithelial cell migration rate for mice after po infection with 1 × 10⁶ pfu virus was compared with that of sham infected mice serving as controls (6). Twelve hours after infection, both groups received tritiated thymidine. At 2, 12, 24, 48, 60, and 72 hr thereafter, the mice were killed and the intestines processed for radioautography. In the infected mice, epithelial cell migration from crypt to villus tip was completed by about 60 hr after infection as compared with about 48 hr for control (sham infected) mice. These results indicate that enteric infection in the mouse with coxsackievirus B5, a human enterovirus, results in a 25% reduction in the rate of epithelial cell division. By contrast, Salmonella infection in the mouse results in an increase in epithelial cell turnover, with cellular extrusion occurring within 24 hr, as compared with 48 hr for noninfected animals (12).

**Discussion**

Hammond and Rosenberg (13) have noted a stimulation of small intestinal mucosal enzymes (sucrase, maltase, aminopeptidase) in neonatal mice following ip infection with coxsackievirus B. A 3-fold elevation in plasma corticosterone was concomitantly also observed. They suggested that the selective enzyme stimulation might be due to the elevation of plasma adrenocorticoid. A direct intracellular effect of the virus, however, could not be excluded. Craighead and Steinke (14) have reported a 3-fold increase in plasma insulin in adult
mice on the fourth day after infection with EMC virus (14). Pancreatic involvement is not uncommon with group B coxsackievirus infection, in adult as well as newborn mice. Recently Webb et al. (15) have demonstrated that coxsackievirus B4 infection in mice resulted in significant histopathologic changes in the pancreas. In extensions of these studies, degranulation of $\beta$ cells have also been observed during the acute stages of such infection. These reports suggest that the increased glucose absorption in our animal model may be due in part to infection-induced elevation of plasma insulin and/or adrenocortical hormones.

Summary

Our findings reveal that intestinal infection with coxsackie B5 results in decreased intestinal epithelial cell division in association with an increase in carbohydrate (glucose) and amino acid (leucine) absorption in the small intestine. These findings are contrasted with those occurring during Salmonella infection, which results in increased intestinal cell division rate but decreased carbohydrate (glucose) absorption.

The changes in intestinal function and physiology that have been described occurred during an asymptomatic viral infection characterized by normal intestinal histology. A reasonable hypothesis is that these pathophysiological changes may be due not only to a variety of local factors but also to hormonal effects induced by systemic spread of coxsackievirus B.

The authors wish to thank Mr. N. Shadoff and Drs. H. Kayne and G.E. Madge for their assistance; Ms. Deborah Goldman, Valerie Verbitzki, Martha Dischinger, and Cathy Zmachinski for their technical assistance.

References


