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Determinants of Diarrhea in Viral Enteritis

The Role of Ion Transport and Epithelial Changes in the Ileum in Transmissible Gastroenteritis in Piglets

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To understand mechanisms of viral diarrhea further, we studied ileal ion transport in vitro in relation to mucosal changes and epithelial differentiation in transmissible gastroenteritis in piglets, an invasive viral enteritis thought to involve mainly proximal intestine. In infected pigs, at the height of diarrhea, short-circuited ileal epithelium failed actively to transport Na⁺ and Cl⁻, and there was a defect of glucose-mediated Na⁺ transport. The Cl⁻ secretory response to theophylline remained intact. Conductance measurements indicate that paracellular permeability may be reduced and transcellular transport may be altered. A mucosal lesion was observed at the time of the transport changes, characterized by villus blunting, crypt hyperplasia, and immature crypt-type enterocytes on the villous epithelium, deficient in disaccharidase and (Na⁺, K⁺)ATPase activity but rich in thymidine kinase. Consideration of the major determinants of diarrhea in this invasive enteritis must take into account not only altered mucosal function and differentiation but also the extent of intestinal involvement, including the ileum, a major site of fluid absorption in the intestine.

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Transmissible gastroenteritis (TGE), an acute invasive coronavirus enteritis of piglets, has been established as a consistent experimental model of viral diarrhea. The TGE virus is thought to invade mainly the epithelium of the proximal intestine, where abnormalities of water and electrolyte transport have been observed in vivo and in vitro at the height of diarrhea. In jejunum in vitro, a specific defect of glucose-stimulated sodium transport has been identified, a defect that coincides with the presence on the villous epithelium of immature crypt-type enterocytes. To assess the role of distal small intestine in the pathogenesis of this viral diarrhea, we measured Na⁺ and Cl⁻ transport across ileal epithelium in vitro at the height of diarrhea and changes in mucosal structure and epithelial differentiation. The ileum is an important site of fluid absorption in the intestine, and we postulated that altered ileal ion transport, a primary driving force for fluid absorption, may be a major determinant of the severity of viral diarrhea.

Methods

Twenty-one 14–16 day old piglets from four litters of conventional York breed swine were studied: eleven received an inoculum of TGE virus; controls were matched-littermates. Infected animals were killed 40 hr after infection, by which time all had moderate-to-severe diarrhea. Sixty centimeters of ileum were removed, starting 5 cm from the ileocecal sphincter: 20 cm for enzyme analyses of isolated villus cells; 15 cm for ion transport studies in vitro; 5 cm for light microscopy; and another 20 cm for enzyme analyses of isolated cells.

Four adjacent segments of ileal mucosa stripped of muscularis were placed in Ussing-type chambers, and steady-state unidirectional mucosa-to-serosa and serosa-to-mucosa Na⁺ and Cl⁻ fluxes were measured in paired tissues in the absence of an electrochemical gradient; an appro-
priate short-circuit current (I_s) was applied to nullify the measured spontaneous transepithelial potential difference (PD). Three consecutive flux study periods were used, and 15 min were allowed for equilibration before each: (a) spontaneous basal fluxes in the absence of glucose; (b) fluxes in the presence of 30 mM glucose; and (c) 10 mM theophylline added after glucose. Unidirectional fluxes (J_{ms} and J_{mn}) were calculated, and net fluxes (J_{net}) were derived from J_{ms} and J_{mn}, expressed as μeq/cm²/hr. Net residual ion flux (net R) of that part of the Is, not accounted for by net movement of Na and Cl, was calculated as I_s - J_{net}Na + J_{net}Cl, where I_s, in μA, was converted to μeq/cm/hr by multiplying by 0.0289 (3.6×10^13/AF), where A = area of mucosa in cm², and F = Faraday constant. Conductance of the tissue (mΩ/cm²) was calculated as I_s/PD×A.

Tissue for light microscopy was fixed in Bouin's fluid, and sections were stained with hemotosylin and eosin. Coded sections were examined by one person without prior identification of the slide, and, as previously described, measurements of villus height and crypt depth, mucosal morphology, and epithelial cell morphology were noted.

Ileal villous enterocytes were isolated selectively with the use of a vibration technique that excludes crypt cells. The presence of intact crypts was confirmed by light microscopy after the isolation procedure. For enzyme analyses, cells were homogenized, stored at −20°C, and protein, using Lowry's method.

Results

Ion Transport In Vitro

Na⁺ flux (Table 1). Under short-circuited conditions, ileal epithelium from TGE-infected animals failed actively to transport sodium either in the presence or in the absence of glucose. In the absence of glucose under basal conditions, J_{net}Na in TGE was negligible and significantly less than the control J_{net}Na, which was absorptive; after administration of 30 mM glucose, J_{net}Na in TGE tissue did not change significantly and remained negligible, in contrast to a significant increase in J_{net}Na in control tissue (P < 0.001). Under basal conditions, unidirectional fluxes (J_{ms}Na and J_{mn}Na) did not differ significantly between TGE and control tissue, but after administration of glucose, J_{ms}Na in TGE was significantly less than the control value and the increment was greater in control tissue (P < 0.02). In the presence of 10 mM theophylline, added after glucose, J_{net}Na in TGE was absorptive, although no significant changes in unidirectional or net fluxes occurred. In control tissue, theophylline caused an increased J_{net}Na (P < 0.01) and a decreased J_{net}Na (P < 0.05), although control J_{net}Na remained significantly greater than TGE J_{net}Na.

Cl⁻ flux (Table 1). In the basal period, J_{ms}Cl, J_{mn}Cl, and J_{net}Cl were lower in TGE compared with control tissue, J_{net}Cl being negligible. Glucose did not significantly alter J_{net}Cl in either tissue, although the increment was greater in control tissue compared with TGE tissue due to an increase in J_{ms}Cl (P < 0.02), which was not observed in TGE tissue. J_{mn}Cl did not change significantly in either tissue after glucose. Theophylline, added after glucose, caused net Cl secretion in both control and TGE tissue, with significant alteration in both flux values (P < 0.001 and P < 0.05, respectively).

Residual flux. A small J_{net} was noted in both tissues and no differences were noted between control and TGE fluxes; both tissues responded similarly to both glucose and theophylline.

Electrical data (Figure 1). In TGE tissue, spontaneous PD was negligible and lower than control PD (P < 0.001). The response to glucose was blunted in TGE compared with the response in controls (P < 0.001), and the PD remained lower (P < 0.001). During the theophylline period, PD in control tissues and PD in infected tissues were not significantly different. Similarly, the Isc (Figure 1 and Table 1) in TGE was negligible, and significantly less than control during the basal period (P < 0.001), and demonstrated a blunted response to glucose when compared with control (P value comparing increments < 0.01). Again, in the presence of theophylline, the differences in Isc between TGE and control tissue were no longer significant. Conductance was lower in TGE during basal and glucose study periods (P < 0.02), although an increment occurred in TGE tissue after administration of glucose (P < 0.02). During the theophylline period, conductance did not differ significantly between the tissues.

Mucosal Structure

Significant structural abnormalities were identified by light microscopy in the ileal mucosa in TGE at 40 hr. All TGE tissue was considered abnormal; mucosal changes varied from mild partial to complete villous atrophy with an abnormal cuboidal epithelium and an increase of inflammatory cells in the lamina propria. In Table 2 the mucosal measurements between control and TGE ileum 40 hr after infection are compared. In TGE mucosa, the villi were shorter, and the crypts were deeper than in control mucosa.
Table 1. Ion Transport across Ileal Mucosa in TGE at the Height of Diarrhea

| Study conditions | No. animals | Na transport | | | Cl transport | | | Residual flux | |
|---|---|---|---|---|---|---|---|---|---|
| | | $I_{ms}$ | $I_{sm}$ | $I_{net}$ | $I_{ms}$ | $I_{sm}$ | $I_{net}$ | $I_{sc}$ | $I_{R_{net}}$ |
| Basal | | | | | | | | | |
| Control | 10 | 5.49 ± 0.41 | 4.04 ± 0.30 | 1.45 ± 0.25 | 5.59 ± 0.30 | 4.26 ± 0.28 | 1.33 ± 0.25 | 0.36 ± 0.04 | 0.22 ± 0.12 |
| TGE | 11 | 4.25 ± 0.52 | 4.34 ± 0.70 | -0.09 ± 0.31* | 3.82 ± 0.22 | 3.32 ± 0.36 | 0.51 ± 0.23 | 0.06 ± 0.06* | 0.63 ± 0.30 |
| | | NS | NS | <0.005 | <0.001 | <0.05 | <0.05 | <0.01 | NS |
| Glucose, 30 mM | | | | | | | | | |
| Control | 10 | 7.76 ± 0.46 | 4.30 ± 0.28 | 3.45 ± 0.38 | 6.65 ± 0.48 | 4.89 ± 0.28 | 1.76 ± 0.27 | 1.71 ± 0.14 | 0.02 ± 0.26 |
| TGE | 11 | 5.78 ± 0.55 | 5.36 ± 0.51 | 0.41 ± 0.35* | 4.62 ± 0.25 | 4.33 ± 0.32 | 0.29 ± 0.18 | 0.63 ± 0.20 | 0.54 ± 0.27 |
| | | <0.02 | NS | <0.001 | <0.001 | NS | <0.001 | <0.001 | NS |
| Theophylline, 10 mM | | | | | | | | | |
| Control | 10 | 7.6 ± 0.50 | 5.13 ± 0.33 | 2.47 ± 0.48 | 5.08 ± 0.32 | 5.96 ± 0.45 | -0.29 ± 0.17 | 1.30 ± 0.10 | -1.46 ± 0.39 |
| TGE | 11 | 6.36 ± 0.42 | 5.61 ± 0.24 | 0.74 ± 0.21 | 4.94 ± 0.35 | 5.44 ± 0.36 | -0.49 ± 0.35 | 0.94 ± 0.11 | -0.89 ± 0.29 |
| | | NS | NS | <0.01 | NS | NS | NS | NS | NS |

* Not significantly different from zero.

b Compares control and TGE.
Figure 1. Potential difference (PD) short-circuit current (Isc), and conductance (Cond) in TGE and control ileal epithelium under basal conditions, with 30 mM glucose, and with 10 mM theophylline. • control; △ infected.

Enzyme Analysis of Villous Enterocytes

Figure 2 depicts the alterations in the enzyme profile of ileal epithelial cells isolated selectively from the villi 40 hr after infection. Compared with control cells, TGE cells were deficient in lactase ($P < 0.001$), sucrase ($P < 0.02$), and Na+, K+ -ATPase activity ($P < 0.001$) but were rich in thymidine kinase activity ($P < 0.005$).

Discussion

Our studies indicate that functional and structural abnormalities can occur in the ileum in this invasive viral enteritis. Severe ileal transport disturbances could contribute to the pathogenesis of the diarrhea. Active sodium transport across ileal epithelium was defective under basal conditions, evidenced by negligible PD, short-circuit current, and net fluxes, and, as previously found in jejunum, thwarted glucose-stimulated Na+ transport. In keeping with these observations, we found deficient Na+, K+ -ATPase activity in cells isolated from ileal villi, indicating a defective active Na+ "pump" in the basolateral membrane. In addition to alterations in transcellular active transport, unidirectional Cl fluxes are reduced, and our electrical data indicate that tissue resistance (reciprocal of conductance) is raised, which implies an alteration in the low-resistance shunt properties of infected tissue. This may indicate a reduction in the size of extracellular pathways and therefore permeability, because shunt conductances of Na, K, and Cl appear to account for a large portion of total conductance in intestinal tissue. These alterations would be expected severely to affect water transport and lead to a net accumulation of water and electrolytes in the lumen, thus contributing to the diarrhea. The intact Cl− secretory response to theophylline shown by the flux data supports previous findings that epithelial adenyl cyclase activity is unchanged in TGE, which suggests that Cl ion secretion mediated by cyclic AMP is not stimulated and does not play a role in the diarrhea.

Previously, using an in vivo marker perfusion technique, we failed to find significant abnormalities.

Table 2. Measurements of Ileal Mucosal Structure in TGE at the Height of the Illness

|          | Crypt (μ) | Villus (μ) |
|----------|-----------|------------|
| TGE (11)a | 258 ± 13  | 80 ± 9     |
| Control (19)a | 130 ± 7 | 284 ± 16   |

$a$ No. of animals.
of ion transport in the distal ileum of TGE-infected pigs. The discrepancy between these earlier in vivo data and the present in vitro findings could be due to (a) a difference between the two studies with respect to the extent and severity of the mucosal lesion or (b) factors affecting ion transport in vivo that cannot be assessed in vitro (e.g., blood and lymphatic flow). We suspect the former is the case, although, of course, we cannot exclude the latter. In the present study, the impact of the infection did extend into the ileum, as indicated not just by alteration of ion-transport phenomena but also by structural and enzyme abnormalities. Our experience of ileal involvement in TGE is similar to that reported by others and is similar to that of other viral enteritides in infant animals. The TGE virus invades primarily the epithelium of proximal small bowel, but invasion can extend for a variable distance to distal intestine. Although the dose of virus and the timing of our experiments was constant, piglets were younger in the present studies than those studied in vivo. It is known that TGE is particularly severe in younger animals, as is the case for other viral enteritides, including human rotavirus in humans. Clearly, the question of the relationship between age-determined severity and the extent of the intestinal lesion needs study.

Villus blunting and crypt hyperplasia were seen in the ileum of TGE-infected animals. The extent and severity of the structural lesion seem variable, even under controlled experimental conditions, and we have shown previously that diarrhea can occur in the absence of a significant light microscopic lesion. The present morphologic data and the enzyme data on cells isolated from villi show a predominance of crypt-type cells in the ileal epithelium after infection, and the transport abnormalities resemble the transport characteristics of normal crypt cells studied in the rat, in which Na⁺ transport is defective compared with that in villous cells and fails to respond to glucose. In addition, these findings support the concept of an ileal epithelium, which, like jejunal epithelium, consists predominantly of undifferentiated immature enterocytes at the stage of severe diarrhea.

The major viral enteritides, including human rotavirus enteritis, appear to have their greatest impact on the upper intestinal epithelium. These studies suggest that consideration of the mechanisms of diarrhea in viral enteritis must take into account not only altered function at a cellular level but also the extent of intestinal involvement. We expect that factors determining the extent and severity of ileal dysfunction may play a major role in the pathogenesis and severity of viral diarrhea.

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