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The Mucosal Lesion in Viral Enteritis
Extent and Dynamics of the Epithelial Response to Virus Invasion in Transmissible Gastroenteritis of Piglets

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In transmissible gastroenteritis (TGE) of piglets, an infection closely resembling human rotavirus enteritis, we studied the timing and extent of epithelial viral invasion along the small intestine after a single oral inoculum of virus; we related these findings to measured alterations in mucosal structure, kinetics, and differentiation, and to previously documented abnormalities of ion transport that occurred at the height of diarrhea. Six and twelve hours after inoculation, before diarrhea, extensive specific viral immunofluorescence and viral particles on electron microscopy were seen in villus but not crypt enterocytes in jejunum, mid-intestine, and ileum. At 24 and 40 hr, when diarrhea was most severe, immunofluorescence was patchy; villus blunting (P < 0.001) and increased crypt depth (P < 0.001) were observed by light microscopy in all segments; radioautographically labeled enterocytes showed accelerated migration and shortened life span; and cells on villi were deficient in sucrase activity (P < 0.001) and rich in thymidine kinase activity (P < 0.005), suggesting relative immaturity. Villus structure recovered by 72 and 144 hr, although deeper crypts (P < 0.001) and accelerated migration still persisted. We conclude that extensive, almost simultaneous direct viral invasion of villus enterocytes can occur along the entire length of the small intestine after a single exposure to virus, and thus can cause shedding of mature villus cells and proliferation and accelerated migration of cells from the crypts. At the height of diarrhea, when enterocyte turnover is maximal, the epithelium consists predominantly of immature virus-free cells which have migrated to the villi in a relatively undifferentiated state.

Transmissible gastroenteritis (TGE), an acute self-limited invasive infection of the small intestine occurring in young pigs, is caused by a Corona virus. Earlier studies of this disease identified specific defects on intestinal transport associated with the presence on villi of poorly differentiated crypt-type enterocytes; these abnormalities coincided with the occurrence of severe watery diarrhea.1,2 Pensaert et al. have shown invasion of the epithelium within 6 hr and shedding of infected cells within 24 hr.3 We suspect that the im-
important defects of intestinal function in TGE develop after virus has been shed from the epithelium.

**Materials and Methods**

Sixty-six 14 to 16-day-old piglets from litters of conventionally weaned York swine received an intragastric inoculum of a standard dose of TGE virus, and 20 uninfected littermates, matched for food intake and age, served as controls. Groups of animals were killed at intervals of 6–144 hr after infection and intestine taken from 3 sites—proximal jejunum (5–15 cm from ligament of Treitz), mid small intestine (10 cm around midpoint), and ileum (5–65 cm from ileocecal sphincter). In the first study at 6, 12, 24, 30, 40, 72, and 144 hr, we examined epithelial viral immunofluorescence (IF) and mucosal structure at all three sites and measured sucrase and thymidine kinase activities in isolated villus cells from the ileal segment. Viral TGE antigen was detected by direct IF, using a fluorescein-conjugated porcine anti-TGE globulin (used for IF diagnosis of field cases, and kindly donated by Dr. A. Gagnon, University of Guelph). Specificity was tested by two-stage inhibition using TGE-inoculated and noninfected cell cultures and TGE-infected jejunal mucosa. In cell cultures, immunofluorescence was seen only in infected cultures; it could be prevented by prior incubation with porcine anti-TGE serum but not gnotobiotic porcine serum. In infected mucosa, bright immunofluorescence seen in enterocytes was completely inhibited by the same specific anti-TGE serum. A much less intense, apparently nonspecific fluorescence in the lamina propria could not be inhibited. Tissue was oriented on wax, fixed in 95% alcohol at 4°C, and embedded in paraffin. After removal of the paraffin, conjugated antiserum was applied to the sections; after incubation in a moist environment for 35 min at 37°C, slides were washed in three changes of phosphate-buffered saline for 10 min, dipped in distilled H₂O, and dried. Sections were mounted in Elvanol (Du Pont Instruments, Wilmington, Del.) and examined with a fluorescence microscope. Epithelial IF was assessed as positive or negative, and its pattern and extent were noted without prior identification of the section. Tissue for light microscopy was fixed in Bouin’s reagent and sections were stained with hematoxylin and eosin (H & E). Coded sections were examined by one person: Villus height and crypt depth were measured by calibrated micrometer (μm), and three representative, well-oriented villi were selected; epithelial injury was semiquantified. Sections for scanning electron microscopy (EM) were washed with cold saline, fixed in 3% gluteraldehyde, processed by the critical-point drying method, and coated with 200 Å of gold-palladium. For transmission EM, sections were fixed in 1% phosphate-buffered OsO₄ and embedded in Epon. Ileal villus enterocytes were isolated selectively with a vibration technique that excludes crypt cells, and the presence of intact crypts was confirmed by microscope examination of tissue after the isolation procedure. For enzyme analyses, cells were homogenized and stored at −20°C for assays of thymidine kinase and sucrase activity.

In the second study, enterocyte migration in proximal jejunum and ileum was measured by autoradiography. Piglets were injected with [³H]thymidine (0.3 μCi/g), and subgroups were killed 6, 12, or 24 hr later so that migration of labeled cells could be measured in relation to mucosal structure. Migration was studied during three stages of the disease (0–24, 36–60, and 120–144 hr) and compared with control data, using six-eight piglets for each study period. Samples of intestine from each site were oriented on wax, fixed in Bouin’s reagent for 12 hr, transferred to 95% alcohol, and embedded in paraffin. Tissue sections (4μm) were

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**Table 1. Epithelial Viral Immunofluorescence During TGE**

| Animals studied | 6 hr | 12 hr | 24 hr | 40 hr | 72 hr | 144 hr |
|-----------------|------|-------|-------|-------|-------|--------|
| Animals with positive fluorescence | | | | | | |
| Proximal jejunum | 6 | 6 | 9 | 11 | 5 | 6 |
| Mid intestine | 6 | 6 | 6 | 0 | 0 | 0 |
| Ileum | 6 | 6 | 8 | 0 | 0 | 0 |
| Distribution of fluorescence | Villus epithelium | Villus epithelium | Villus tips | Occasional villus tip |

* Continuous immunofluorescence (IF) over most villus tips. b Extensive continuous IF over at least 50% of most villi. c Sporadic IF on a few villi.
dipped in Kodak NTB-3 emulsion, exposed for 3 wk at 4°C, developed in Kodak Dektol, and stained with H & E. Coded sections were examined with a calibrated eyepiece by one person, who selected from each section (excluding areas over Peyer's patches) 10–30 longitudinally well-oriented crypt-villus columns with the lumen in continuity. Crypt-villus column height and the distance of foremost labeled cells (FLC) from crypt base were measured; in addition, to allow for changes in column height, the mean position of FLC was expressed as a percentage of column height. Migration rates were expressed as change in position of FLC per hour, and the mean enterocyte transit time (time taken for label to reach the villus tips) was calculated for each stage of the disease.

Results were analyzed with Student's t-test. Analyses of enzyme data were based on the assumption of a logarithmic distribution for the data, and the results were expressed as antilogarithms.

### Results

**Viral Immunofluorescence**

Specific epithelial viral IF was observed consistently in proximal jejunum, mid small intestine, and ileum during TGE. Data from 5-14 animals at each time interval; expressed as mean villus height and mean crypt depth (µm) ± 1 SEM. Compared with control, P < 0.001.

| Enzyme Activities of Isolated Ileal Villus Enterocytes during TGE |
|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Enzyme activity      | Control          | 12 hr            | 24 hr            | 40 hr            | 72 hr            | 144 hr           |
| n                    | 14               | 8                | 9                | 11               | 5                | 6                |
| Sucrase: Mean        | 23.4             | 24.5             | 4.9              | 3.7              | 8.9              | 11.55            |
| Range                | 20.0–27.5        | 18.6–32.4        | 3.6–6.6          | 5.1–4.9          | 5.5–13.9         | 11.0–12.6        |
|                      | NS < 0.001 < 0.001 | NS < 0.001 | NS < 0.05   | NS < 0.05 | NS < 0.05   |
| Thymidine kinase:    |                  |                  |                  |                  |                  |                  |
| Mean                 | 12.2             | 23.9             | 35.5             | 27.8             | 23.6             | 15.4             |
| Range                | 10.5–14.4        | 6.4–36.3         | 26.9–46.7        | 22.0–32.0        | 18.8–29.7        | 10.4–19.2        |
| p<sup>a</sup>        |                  |                  |                  |                  |                  |                  |

Table 2. Enzyme Activities<sup>a</sup> of Isolated Ileal Villus Enterocytes during TGE

<sup>a</sup> Results expressed as antilog mean and range of 1 SEM, where n = number of animals. Sucrase was measured as µmoles of glucose per minute per milligram protein, and thymidine kinase as pmoles of thymidine phosphate per minute per milligram protein.

<sup>b</sup> Compared with control; NS = not significant.
and ileum of all animals studied at 6, 12, and 24 hr after infection but was rare at 40, 72, and 144 hr (Figure 1 and Table 1). In control tissue, nonspecific pale yellow plasma fluorescence was observed in the lamina propria. The specific apple-green viral immunofluorescence that appeared in the apical cytoplasm of villus enterocytes of infected tissue, particularly toward the tips (Figure 1), was not seen in the crypts and appeared to be confined to the villus epithelium. At 6 hr, IF was observed in most of the villus epithelium of proximal and mid small intestine but was concentrated in the villus tips in the ileum; in one piglet it was limited to the proximal segments only (Table 1). At 12 hr, IF appeared equally extensive in all segments, but by 24 hr it was inconsistent, patchy, and confined to occasional blunted villus tips.

Mucosal Structure

Significant structural abnormalities were observed on light microscopy in all three intestinal segments—after the time when viral IF was maximal. In infected pigs (Figure 2), at 24 and 40 hr, the villi were shorter, and crypts were deeper than controls \((P < 0.001)\), and by 72 and 144 hr the villi were recovering although crypts remained hyperplastic. At 40 hr, the mucosal lesion in all three segments varied from mild partial villus atrophy to flattening of the mucosa (Figures 3 and 4), the villus epithelium was cuboidal with a poorly defined brush border, crypts were hyperplastic, and the lamina propria was infiltrated with inflammatory cells (Figure 3B).

By electron microscopy at 12 and 24 hr and in an occasional cell in one animal at 40 hr, viral particles were not seen in crypt epithelium at any stage or in any tissue taken at later stages of infection. At 24 and 40 hr, villus surface enterocytes seen by electron microscopy and compared with controls had irregular sparse microvilli, apical nuclei, fewer organelles of the apical cytoplasm (Figure 6), and intraepithelial lymphocytes were observed.

Enzyme Activities in Ileal Villus Enterocytes

Sequential changes in sucrase and thymidine kinase activities in villus cells isolated 12, 24, 40, 72, and 144 hr after infection are shown in Table 2. By 24 and 40 hr, sucrase activity was significantly less than in cells from controls, and thymidine kinase activity was significantly greater. By 144 hr, thymidine kinase activity did not differ from controls, although the villus cells were still sucrase deficient.

Cell Migration

The position of foremost-labeled cells in crypt-villus columns, expressed both as distance from crypt base and as a percentage relative to column height, are shown in Table 3. In both jejunum and ileum, mean distance from crypt base was significantly greater than the corresponding control measurement 6 hr after labeling in the TGE-infected groups studied at 36 and 120 hr after infection \((P < 0.001)\). In those given label 36 hr after infection, the foremost label at 6 and 12 hr was significantly further from the crypt base than in those studied earlier \((0-24 \text{ hr})\) and later \((120-144 \text{ hr})\) in the disease \((P < 0.001, \text{ respectively})\); in fact, at this stage of the disease the label had reached the tips of villi within 24 hr of injection. When FLC position was expressed as a percentage of column height, allowing for ob-
Figure 4. Scanning electron micrographs of distal small intestine (× 50). A. Section from control showing slender finger-like villi. B. Section from TGE-infected piglet at 40 hr. Loss of villi is complete over the folds. Surface openings of crypts appear as pits on the irregular surface.

served changes in column height, similar differences were observed in both segments (Table 3). As indicated in the table, these data are reflected in the migration rates, expressed as distance traveled by the foremost-labeled cell per hour. In TGE, migration rates greatly exceeded control values, particularly in the 36–60 hr infected group (TGE, 16.7 and 12.7 vs. control, 8.9 and 7.0 μ/hr in jejunum and ileum, respectively). The estimated time taken for foremost labeled cells to reach the villus tips was markedly reduced in both jejunum and ileum during TGE, related both to a significant increase in migration as previously indicated, and to a significant reduction in total crypt-villus column height at certain stages of infection (Table 3). For example, the enterocyte transit time or life span was 55 hr in control jejunum, compared with 18 hr for cells labeled 36 hr after infection in TGE. During this study period, the measurements of column height were all significantly less than control measurements (P < 0.001).

Discussion

The present observations help to clarify the relationship between viral invasion and the functional defect characterized in our earlier studies. The abnormalities of ion transport observed in jejunum and ileum at the height of diarrhea in TGE are clearly a response to direct visual invasion of these intestinal regions, but they occur after viral IF has disappeared from the mucosa. Our observations support earlier data from Pensaert et al.7 on the time of appearance and disappearance of TGE IF in the jejunum. They also show that infection of the ileum coincides with jejunal infection, and, therefore, it is unlikely to be a consequence of exposure to virus shed from infected upper gut.

The TGE virus invaded the villus but not the crypt epithelium of the small intestine within hours of administration. Because the virus is absent from the epithelium of the resulting mucosal lesion, we assume that the infected cells have been shed at that stage, replaced by relatively undifferentiated enterocytes as judged by structural and enzymatic criteria, and not reinfected by virus. As others have noted,7 villus cells seem particularly susceptible to invasion by the TGE virus. Many factors could be involved in this apparent resistance of less differentiated crypt-type enterocytes to virus invasion. On electron microscopy, we observed viral particles actually traversing intact microvilli of mature epithelial cells; perhaps a brush-border receptor is synthesized as enterocytes differentiate in the course of their migration from crypt to villus tip.

As infected villus cells are shed, our kinetic and enzyme data show that the epithelium proliferates and migration of cells from the crypts accelerates. The pathogenesis of this proliferation is unknown,
|                  | Studied TGE (hours after infection) | Jejunum |          | TGE (hours after infection) | Ileum |          |
|------------------|------------------------------------|---------|----------|-----------------------------|-------|----------|
|                  | Control 0-24 36-60 120-144         |         |          | Control 0-24 36-60 120-144 |       |          |
|                  |                                    |         |          |                              |       |          |
| Crypt-villus column height (μm) |                                    |         |          |                              |       |          |
| 6                | 609 ± 12 493 ± 8 370 ± 8           | 407 ± 5 | 408 ± 3  342 ± 6  411 ± 7 |
| 12               | 494 ± 6 396 ± 9 386 ± 9           | 412 ± 5 | 388 ± 8  355 ± 7  415 ± 5 |
| 24               | 483 ± 6 332 ± 5 402 ± 8           | 416 ± 5 | 289 ± 7  394 ± 6  408 ± 4 |
| Distance of FLCb from crypt base (μm) |                                    |         |          |                              |       |          |
| 6                | 77 ± 2 78 ± 4 131 ± 4c            | 71 ± 2  71 ± 3  194 ± 5c  134 ± 4c |
| 12               | 126 ± 4 156 ± 5c 209 ± 4c         | 102 ± 4 | 112 ± 3  270 ± 10c  206 ± 6c |
| 24               | 230 ± 4 302 ± 5c 334 ± 6c         | 198 ± 3 | 256 ± 5c  Villus tips  351 ± 4c |
| Position of FLCb (% of column height) |                                    |         |          |                              |       |          |
| 6                | 16.0 ± 0.6 56.1 ± 2.7c 36.8 ± 0.5c | 18.0 ± 0.8 17.6 ± 0.5  56.9 ± 1.9c  32.4 ± 0.9c |
| 12               | 25.0 ± 0.9 81.6 ± 2.5c 50.9 ± 5.0c | 25.7 ± 0.6 29.9 ± 0.6c  76.8 ± 1.0c  49.8 ± 1.7c |
| 24               | 49.5 ± 0.8 91.1 ± 0.6c 79.7 ± 0.6c | 47.5 ± 0.9 88.4 ± 16c  86.0 ± 1.1c |
| Migration rate (μm/hr)cd | 8.9 12.4 16.7c 11.3  | 7.0 10.3 12.7c 12.1 |
| Enteroocyte transit time (hr)ef | 55 26 18 32  | 56 27 18 34  |

a Each value represents the mean ± SE of a minimum total of 30 measurements (av = 48) from one to three animals.
b FLC (foremost labeled cell).
c FLC position: compared with control. P < 0.001.
d Migration between 12 and 24 hr. except: * 6–12 hr.
e Estimated time for FLC to reach villus tip or 100% of crypt-villus column.
but a negative feedback from mature villus epithelium has been postulated in normal mucosa. As the replacement enterocytes migrate, they have a shortened life span and at the peak of disease fail to differentiate fully. The findings of defective differentiation and shortened life span strongly support the concept that, at the height of diarrhea, the renewed epithelium in TGE consists predominantly of immature cells. Clearly, the abnormalities in transport function which reflect an altered balance between immature crypt and mature villus cells cannot be attributed directly to the presence of virus.

Several acute viral intestinal infections affecting the young of various species appear to follow the sequence just outlined for TGE, with invasion and desquamation of mature villus cells, leading to a mucosal lesion and to diarrhea coincident with epithelial regeneration. These include coronavirus enteritis of dogs and calves and rotavirus enteritis of piglets, lambs, and calves. Our recent experience with human rotavirus enteritis induced in piglets leads us to believe that a similar sequence of events may occur in the human condition. Because this epithelial response is central to the pathogenesis of diarrhea caused by viral invasion, factors that influence mucosal renewal such as age, drugs, and nutrition may be significant determinants of recovery from these diseases.

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Figure 6. Transmission electron micrographs of surface enterocytes from mid small intestine (X 6,500). A. From control piglet. The nucleus (Nu) is situated in the basal portion of the cytoplasm. Cell organelles are normal and abundant, and the brush border is regular. B. From TGE-infected piglet, 24 hr after infection, showing a cryptlike cell in the surface epithelium. The nucleus is apical, rough endoplasmic reticulum is sparser than in control, and the brush border is short irregular. An intraepithelial lymphocyte (Ly) is seen.

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