Comparative Gastroenterology

Early Pathogenesis of Colitis in Neonatal Pigs Monocontaminated with Escherichia coli

Fine Structural Changes in the Colonic Epithelium

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Escherichia coli was found attached to and envacuolated within colonic columnar epithelial cells from 24 to 144 hr after monocontamination of neonatal pigs. Cellular changes were minimal until edema of the lamina propria developed 144 hr after monocontamination. Intracellular lipid was increased after bacterial penetration, but lipid was also present in the colonic epithelium of neonatal germfree pigs. Cellular degeneration did not appear to result directly from bacterial invasion of the cell, but rather, from subsequent edema of the subepithelial lamina and impaired circulation, perhaps as the result of intravascular endotoxin.

The experimental condition described may provide a basis for elucidating the pathogenesis of ulcerative colitis and/or regional enteritis of the colon.

Escherichia coli-induced colitis, as a separate disease entity in the neonatal pig, has received little consideration. Colitis in young pigs has been reported (1, 2, 3) but the morphologic lesions described have been minimal and the pathogenesis has not been elucidated.

The colonic epithelium has been implicated in the pathogenesis of ulcerative colitis due to the presence of antibodies to fetal colonic epithelium in the serum of children with ulcerative colitis (4). However, epithelial changes may be secondary and due to microvascular insufficiency (5).

There are no ultrastructural studies on the role of the colonic epithelium in regional enteritis of the colon. Hawk et al (6) expressed the opinion that the effect of the etiologic factor or factors is directed to the structures of the submucosa in regional enteritis of the colon. Likewise, Meadows and Batsakis (7) reported that the earliest detectable lesions of regional enteritis of the small intestine was submucosal edema.

E coli attachment to and penetration into the ileal epithelium of colostrum-deprived monocontaminated pigs produced a mild enteritis (8). These same pigs were also observed to have a mild colitis. Since these preliminary observations indicated that the colonic epithelium was subject to penetration by E coli and since in advanced cases of E coli enteritis (9), edema has been observed in the mesocolon concomitant with diarrhea, it was felt that ultrastructural alterations in the colonic epithelium must be present.
In addition, Law (10), in reviewing the multiple facets of regional enteritis in man, indicated that microorganisms have not been meaningfully implicated in the pathogenesis of this disease. This experimentally monocontaminated neonatal pig appeared to be an opportune model for the study of colitis produced by a single etiologic agent. This study, therefore, characterizes the fine structural changes in the colonic epithelium of neonatal pigs monocontaminated with \textit{E. coli} (055B5). A subsequent paper will describe the ultrastructural changes in the circulatory compartments of the lamina propria and submucosa (11).

**MATERIALS AND METHODS**

The colons of 22 pigs monocontaminated with \textit{E. coli} (055B5) were examined at intervals after exposure. All pigs were caesarean-derived at 112-115 days of gestation; within 1-2 hr after birth they were dosed intragastrically with 1 g \textit{E. coli}/body weight suspended in 10 ml sterile saline. Bacteriologic plate counts revealed 10⁹ organisms/ml. The dosed pigs were maintained in sterilized plastic isolators and fed a synthetic milk diet. Pigs were sacrificed at approximately 24, 48-60, 96-100 and 144 hr after dosing. The pigs sacrificed at the 48-60 and 96-100 hr intervals in many instances were in the early stages of expiration. The 144-hr post exposure pigs were seemingly healthy in spite of very loose stools. The colons of newborn and 6-day-old germfree pigs were examined as controls. All pigs were anesthetized and prepared for surgical removal of the specimens for subsequent light and electron microscopy.

**Tissues for light microscopy.** Specimens of the spiral colon, both ascending and descending, were collected in neutral buffered 10% formalin. Paraffin sections were cut and stained with either Gram's stain, periodic acid-Schiff reagent or hematoxylin-eosin. These tissues were examined by light microscopy for pathologic changes and/or the presence of \textit{E. coli}. Frozen sections were stained with oil red 0 and examined for intracellular fats.

**Tissues for electron microscopy.** Specimens from the distal tip of the spiral colon were removed, spread on gauze saturated with cold saline, opened along the mesenteric border and placed in cold (4°C) 2.5% glutaraldehyde buffered with 0.2 M sodium cacodylate or s-collidine. Tissues were generally fixed overnight in the refrigerator and processed as previously described (8). Some tissues were held 2-3 months in glutaraldehyde before processing. In some instances, tissues fixed in formalin were washed with 0.2 M sodium cacodylate for 2 hr and processed as above. One-micron sections were cut, mounted on glass slides, stained with 1% alkaline toluidine blue and examined by light microscopy for bacterial attachment. Thin sections (600-800 Å) were cut, mounted on unsupported copper grids and stained with lead citrate or alternately with uranyl acetate.

**RESULTS**

\textit{E. coli} were observed adhering to colonic epithelium at all sampling intervals studied (Fig 1, 2). Microbial attachment to the apical ends of the columnar cells occurred most frequently on the luminal epithelium (epithelium on the surface, not in the crypts). Attachment occurred with decreasing frequency in the midportion and base of the crypts. At the most common site of attachment, the microvilli were in varying degrees of exfoliation and bacteria were closely apposed to the denuded apical plasma membrane (Fig 3). There was no obvious predilection for cell types; attachment appeared to be random. At 24 hr after infection, affected cells were paler and the apical plasma membrane bulged into the gut lumen. Few intracellular bacteria could be found at this time. Once \textit{E. coli} had attached itself, other \textit{E. coli} accumulated at this site until several layers of organisms occurred on the surface of the epithelium. Dividing organisms were common (Fig 1, 3). \textit{E. coli} which were free in the intestinal lumen, but in close proximity to the colonic epithelial cells, had portions of microvilli attached to the cell wall of the bacteria (Fig 4).