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NOTES

Infection of Gnotobiotic Pigs with an *Escherichia coli* O157:H7 Strain Associated with an Outbreak of Hemorrhagic Colitis

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Gnotobiotic pigs inoculated with an *Escherichia coli* O157:H7 strain isolated from a human with hemorrhagic colitis developed anorexia, lethargy, and watery diarrhea. Bacteria diffusely colonized the cecum and colon surfaces and the crypt epithelium. At bacterial attachment sites, microvilli were effaced, and epithelial cells were irregularly shaped, rounded, or detached. Submucosa, lamina propria, and mesentery were markedly edematous and contained many inflammatory cells.

Escherichia coli O157:H7 has been associated with outbreaks and a number of sporadic cases of hemorrhagic colitis in the United States and Canada (8–10; W. M. Johnson, H. Lior, and G. S. Bezanson, Letter, Lancet i:76, 1983). The illness has been characterized by severe crampy abdominal pain and watery diarrhea followed by grossly bloody diarrhea (10). Hemolytic/uremic syndrome has been cited as a complication of the illness (2). The organism does not produce heat-stable or heat-labile enterotoxin and is nonenteroinvasive as determined by the Sereny test (14), but it does produce a Shiga-like cytotoxin (A. D. O'Brien, T. A. Lively, M. E. Chen, S. W. Rothman, and S. B. Formal, Letter, Lancet i:702, 1983). Infant rabbits inoculated with *E. coli* O157:H7 developed a watery, nonbloody diarrhea (Hemorrhagic Colitis Task Force, Letter, Lancet i:702–703, 1983). However, no histopathologic studies were performed on infected animals. Chickens (1 day old) inoculated with *E. coli* O157:H7 remained healthy, but the bacteria induced mild, transient cecal mucous membrane lesions (1). Attachment, microvillous effacement, and penetration of the cecal surface epithelium by the organisms were observed. The pathogenicity of human-derived enteropathogenic *E. coli* strains for gnotobiotic pigs has been investigated previously (6, 13). Attachment to and effacement of colonic and cecal microvilli were observed, suggesting that the newborn gnotobiotic pig is a useful animal for studying such enteropathogenic *E. coli*. The purpose of the present study was to determine whether *E. coli* O157:H7 is pathogenic for neonatal gnotobiotic pigs and, if pathogenic, to characterize the lesions resulting from infection.

Fourteen piglets, derived by closed hysterotomy and housed in germfree isolators, were divided into two groups and inoculated orally at 1 day of age with approximately 10^{11} CFU of *E. coli*. Eight piglets received *E. coli* O157:H7 strain EDL931. Six piglets received nonpathogenic *E. coli* O101:K28 strain G58-1. Strain EDL931 (obtained from George K. Morris, Centers for Disease Control, Atlanta, Ga.) was isolated from a patient with hemorrhagic colitis in an Oregon outbreak described elsewhere (10). Strain G58-1 (obtained from Werner K. Maas, New York University, N.Y.) is a tetracycline-sensitive derivative of a strain isolated from swine feces. It possesses no plasmids, produces no enterotoxins as determined in piglet intestinal loops, and is nontoxic to Vero cells (unpublished data).

Piglets were examined several times daily for diarrhea and other clinical signs. One piglet from each group was killed 1 day after inoculation. Three piglets from each group were killed 2 days after inoculation. Two piglets from the group receiving strain EDL931 and one piglet from the group receiving strain G58-1 were killed on each of days 3 and 4 after inoculation. Sections of duodenum, jejunum, ileum, cecum, spiral colon, and rectum were removed, immediately rinsed with 10% neutral buffered Formalin, and immersed in Formalin or Karnovsky's fixative (3).

Formalin-fixed tissues were processed by standard methods and stained with hematoxylin and eosin for light microscopy. Karnovsky-fixed tissues were washed in 0.2 M cacodylate buffer, postfixed in 1% OsO_4, rinsed in buffer, dehydrated in graded ethanols, and embedded in Epon-812 resin. Thick sections were cut, stained with toluidine blue, and examined by light microscopy. Ultrathin sections were cut from selected blocks, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope at 60 kV. Karnovsky-fixed tissues for scanning electron microscopy were rinsed in three changes of 0.2 M phosphate buffer at room temperature and washed for 8 h in each of three changes of distilled water. Tissues were then postfixed with thiocarbohydrazide and OsO_4 (4), dehydrated as reported elsewhere (5), and critical-point dried in liquid carbon dioxide. Dried specimens were attached to aluminum stubs with silver conducting paint and examined with a scanning electron microscope at an accelerating voltage of 15 kV.

Pigs inoculated with the nonpathogenic control strain G58-1 remained healthy, and their intestines were normal as determined by gross and histologic examination. The microvillous morphology was unaltered even though there

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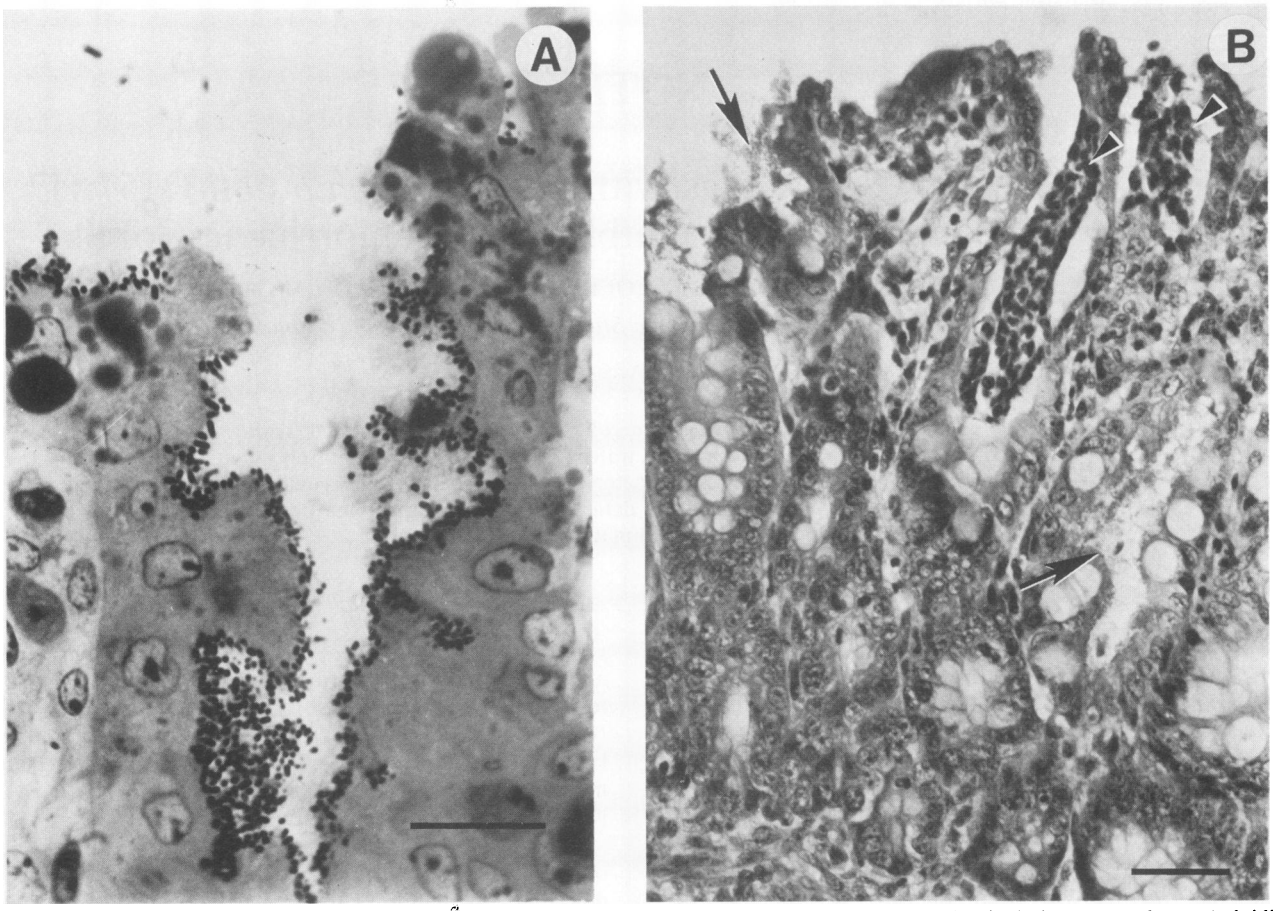

FIG. 1. (A) Irregularly shaped, cuboidal to low columnar crypt-neck cells with bacteria adherent to luminal plasma membrane (toluidine blue stain). Bar, 5 μm. (B) Glandular crypts containing neutrophils (arrowheads) and enterocytes colonized by bacteria. Indistinct layers (arrows) were identified as bacteria at higher magnifications. (Hematoxylin and eosin stain.) Bar, 10 μm.

were foci of adherent bacteria in the ileum, cecum, and spiral colon of two control pigs.

Pigs inoculated with strain EDL931 were anorectic and lethargic by 2 days after inoculation. Their feces were more fluid than those of control pigs but did not contain grossly visible blood. At necropsy, mild to marked edema was observed in the mesentery of the spiral colon in all of the piglets inoculated with this strain. As determined semiquantitatively by culturing on blood and Tergitol-7 agar, the ilea and colons of both G58-1-and EDL931-inoculated pigs were colonized by large numbers of *E. coli* with colony types characteristic of the respective inoculation strains.

Intestinal tracts of pigs inoculated with strain EDL931 had bacteria diffusely adherent to epithelial cells in the cecum and spiral colon and focally adherent in the ileum and rectum. Both crypt and surface epithelia were colonized. At 1 day after inoculation, bacteria colonizing crypts were attached to crypt-neck cells, but by 4 days after inoculation the colonization of the cecum and colon involved the entire crypt length. The ileal colonization involved the sides and base of villi as well as glandular crypts.

Epithelial cell alterations associated with adherent bacteria were the same in the ileum and large intestine. Epithelial cells in areas of attached bacteria were cuboidal to low columnar with an uneven mucosal border (Fig. 1A). There were cytoplasmic blebs and detached epithelial cells with adherent bacteria in intestinal and crypt lumina. Inflammation, characterized by neutrophils in intestinal crypts, epithelium, and lamina propria, was evident in the cecum and colon 1 day after inoculation (Fig. 1B). Three days after inoculation, submucosa and lamina propria in these tissues were markedly edematous and contained many lymphocytes, macrophages, and neutrophils.

Ceca of EDL931-infected piglets viewed with the scanning electron microscope showed that many bacteria were attached to epithelial cells. Infected cells had few disoriented or elongated microvilli, were rounded, and bulged into the intestinal lumen (compare EDL931-infected ceca with G58-1-infected ceca [Fig. 2]).

Intestines of EDL931-inoculated piglets viewed with the transmission electron microscope showed that randomly arranged bacteria were intimately associated with apical plasma membranes of crypt and surface epithelial cells. At attachment sites, microvilli were effaced or sometimes fused, and the number of microvilli between bacteria was reduced. Bacteria were frequently associated with projections ("pedestals") or invaginations ("cups") of plasma membrane. The terminal web of infected cells was absent or modified to electron-dense fibrillar deposits (Fig. 3).

Results of the experiments reported here indicate that strain EDL931 is an attaching and effacing *E. coli* and produces lesions similar to those caused by other entero-

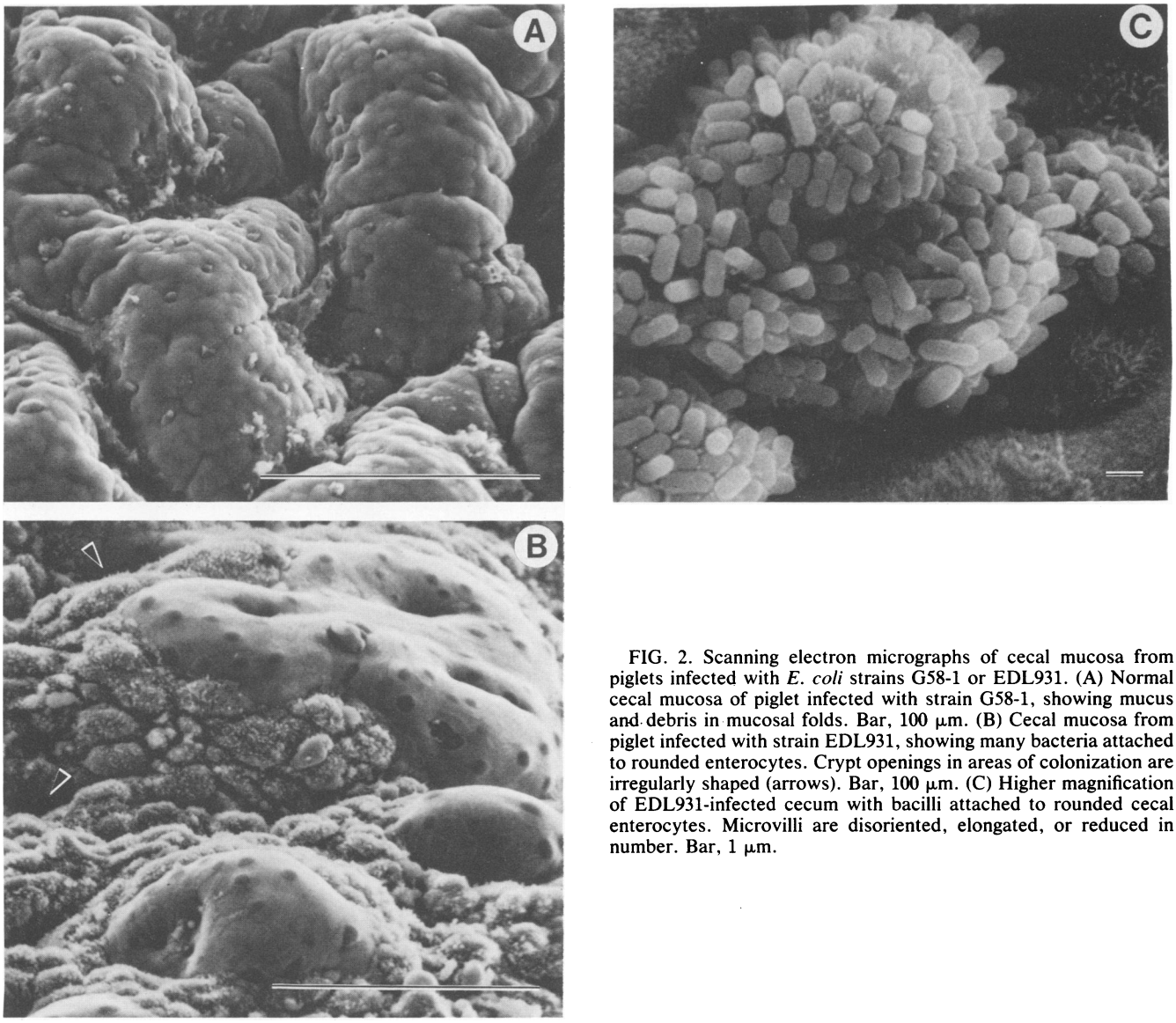

FIG. 2. Scanning electron micrographs of cecal mucosa from piglets infected with *E. coli* strains G58-1 or EDL931. (A) Normal cecal mucosa of piglet infected with strain G58-1, showing mucus and debris in mucosal folds. Bar, 100 μm. (B) Cecal mucosa from piglet infected with strain EDL931, showing many bacteria attached to rounded enterocytes. Crypt openings in areas of colonization are irregularly shaped (arrows). Bar, 100 μm. (C) Higher magnification of EDL931-infected cecum with bacilli attached to rounded cecal enterocytes. Microvilli are disoriented, elongated, or reduced in number. Bar, 1 μm.

pathogenic *E. coli* strains of human origin (6, 11, 12). However, strain EDL931 appears to affect crypt epithelium more consistently and severely.

The attaching and effacing *E. coli* strains studied by Moon et al. (6) in gnotobiotic pigs were inconsistent in producing clinical illness, were more focal in colonization and lesion production, and stimulated a less pronounced inflammatory response than did strain EDL931. Differences in virulence for pigs between strain EDL931 and these other strains may be the result of more diffuse colonization by strain EDL931. Alternatively, it may be that strain EDL931 is more toxigenic. Strain EDL931 produces large amounts of Shiga-like toxin (A. D. O'Brien, T. A. Lively, M. E. Chen, S. W. Rotham, and S. B. Formal, Letter, Lancet **i**:702, 1983). One strain (E851/71), studied by Moon et al. (6) and O'Brien et al. (7), produced only modest amounts of Shiga-like toxin. The other strains were not tested.

The clinical features of *E. coli* O157:H7 colitis in pigs were similar to those observed in humans except that blood was not grossly visible in feces. However, it should be noted that the stools of infected humans also may be free of grossly evident blood (9). The location of the infection in pigs was

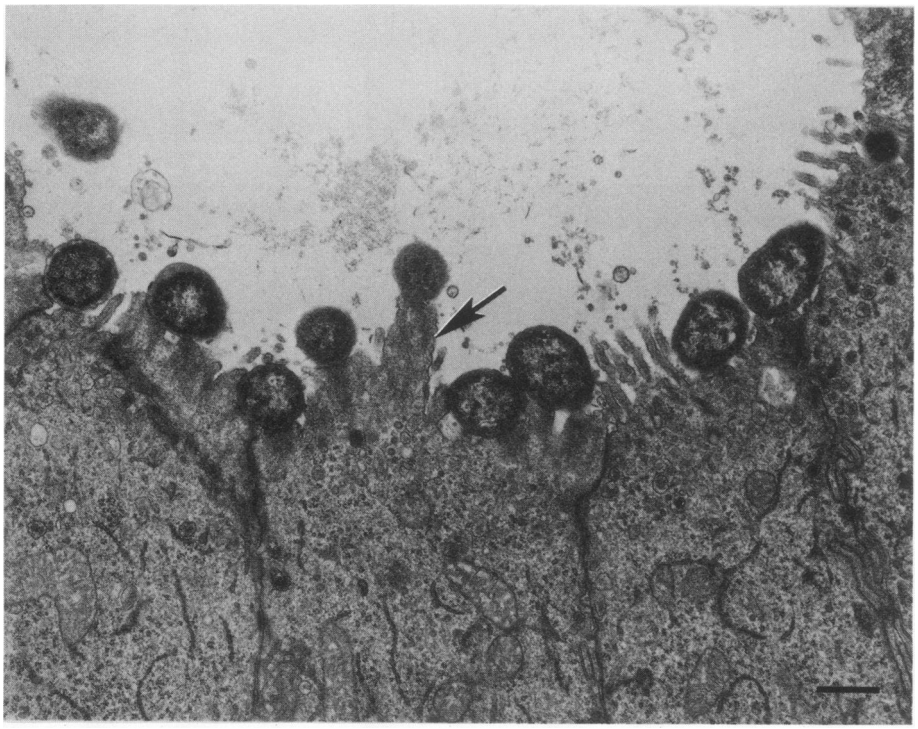

FIG. 3. Transmission electron micrograph of cecal crypt epithelium from a gnotobiotic piglet infected with *E. coli* EDL931. Cytoplasmic projections and invaginations and electron-dense modifications of terminal web are associated with *E. coli* attached to epithelial cells. Microvilli are effaced and fused (arrow). Bar, 1 μm.

the same as that suggested by radiography in humans (10), and edema of colonic mesentery was present in both humans and pigs. Based on these findings, it is suggested that the 1-day-old gnotobiotic pig is a suitable animal for studying *E. coli* O157:H7 colitis of humans.

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