CONCISE COMMUNICATION

A Monkey Model for Enterohemorrhagic *Escherichia coli* Infection

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Adult *Macaca radiata (n = 22)* were infected intragastrically with 10⁷ *Escherichia coli* O157: H7 strain 84-01, which produces Shiga toxins 1 and 2. Clinical symptoms and bacterial excretion were documented in each monkey for a specified time period before they were killed. At necropsy, samples were obtained for culture and histologic and ultrastructural examination. Seventeen monkeys had diarrhea: *E. coli* O157 was isolated from postinfection stool samples from all monkeys and from autopsy cultures for 14 of 22 monkeys. Histologic examination showed attaching-effacing lesions, which appeared at 12 h and persisted for 7 days, in 12 monkeys. Widening of the intercellular spaces, degeneration and vacuolization of the epithelial cells, epithelial tufting, extrusion of epithelial cells, and neutrophilic infiltration were characteristic features seen in 20 of the 22 infected monkeys but not in 4 control monkeys. This monkey model closely parallels the early stages of the disease produced by *E. coli* O157:H7 and would be useful in the further study of pathogenic mechanisms and prevention methods in enterohemorrhagic *E. coli* infections.

Enterohemorrhagic *Escherichia coli* (EHEC) cause a range of human illnesses, from nonbloody diarrhea to hemolytic-uremic syndrome and neurologic symptoms [1]. The intimate adherence of EHEC to the intestinal epithelium produces characteristic attaching-effacing (A/E) lesions in the intestines of gnotobiotic piglets, infant rabbits, and chickens [2–4]. These lesions are not seen in a mouse model or in human infections [5–8]. EHEC isolates also produce Shiga toxins 1 and 2 (Stx 1 and 2), which are bacteriophage-encoded potent cytotoxins that induce microvascular changes in vivo and are paralytic and lethal for mice [9].

In the last decade, several animal models have been developed for EHEC pathogenic studies, including streptomycin-treated mice [4], chickens [2], infant rabbits [1], gnotobiotic piglets [10], and calves [11]. However, these animals lack normal adult gut flora, and EHEC can be nonpathogenic in older animals. This study was undertaken to see whether the clinical, bacteriologic, and pathologic changes characterizing human EHEC infections could be produced in adult monkeys with normal gut microflora.

Materials and Methods

*E. coli strain. E. coli* O157:H7 strain 84-01, which produces both Stx 1 and 2, was used in this study. Bacteria were stored at −70°C in brucella broth with 10% glycerol.

Preparation of infective dose. Bacteria were grown overnight at 37°C in Luria-Bertani broth and then were centrifuged. The pellet was resuspended in 10 mL of PBS, to give a final infective dose of 10⁷ organisms/mL.

Experimental monkeys. Wild-caught *Macaca radiata* were screened for bacterial pathogens and parasites, including *Cryptosporidium* species. Samples were cultured, and adherence assays and toxin testing were done to identify bacterial enteric pathogens, but screening results were negative. Parasite screening identified helminths (mainly *Enterobius* species ova) and protozoa (*Giardia* species and *Balantidium coli*), and affected monkeys were administered 750 mg pyrantel pamoate and 100 mg metronidazole, which eliminated parasites in most monkeys. Quantitation of anaerobic and aerobic flora in 4 monkeys showed that recovery to premetronidazole levels occurred in 4 days.

Monkeys who had not had diarrhea for 2 weeks were experimentally infected when 2 stool examinations had negative results for pathogens and when 2 weeks had elapsed since treatment. Of 26 experimental monkeys, 4 served as controls and were infected with *E. coli* KM 124, an isolate obtained from an asymptomatic rural South Indian patient whose assay results were negative for diarrheogenic *E. coli*, and 22 monkeys were infected with O157: H7 strain 84-01.

Infection. The monkeys were anesthetized by intramuscular injection of ketamine hydrochloride (4 mg/kg). Gastric acidity was neutralized with a 10-mL dose of 10% sodium bicarbonate, which was administered via an orogastric tube. Ten minutes later, the infective dose was administered through the same tube. Infected monkeys had diarrhea: *E. coli* O157 was isolated from postinfection stool samples from all monkeys and from autopsy cultures for 14 of 22 monkeys. Histologic examination showed attaching-effacing lesions, which appeared at 12 h and persisted for 7 days, in 12 monkeys. Widening of the intercellular spaces, degeneration and vacuolization of the epithelial cells, epithelial tufting, extrusion of epithelial cells, and neutrophilic infiltration were characteristic features seen in 20 of the 22 infected monkeys but not in 4 control monkeys. This monkey model closely parallels the early stages of the disease produced by *E. coli* O157:H7 and would be useful in the further study of pathogenic mechanisms and prevention methods in enterohemorrhagic *E. coli* infections.

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The experimental protocol was approved by the institutional ethics committee of the Christian Medical College and Hospital.

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Monkeys were housed in cages in a separate room in the animal house and were observed twice daily. Stool samples were collected for culture once (for monkeys without diarrhea) or twice (for monkeys with diarrhea) daily.

**Autopsy:** Two monkeys were killed at each of 8 postinfection time points (6 and 12 h and days 1–5 and 7), and 3 monkeys were killed 9 and 12 days after infection. Two control monkeys were killed on postinfection days 1 and 3.

The monkeys were assigned to specific infection durations before the start of the experiment, and no changes were made during the course of the study. At necropsy, samples were collected for culture and biopsy from the stomach, duodenum, jejunum, ileum, cecum, proximal colon, mid-colon, distal colon, and rectum. Tissue from the mesenteric nodes, kidney, liver, spleen, and lungs was also collected.

**Bacteriologic examination.** Quantitative culture of stool and culture of autopsy material was done on sorbitol-MacConkey agar. Sorbitol-negative E. coli colonies were tested by slide agglutination of a saline suspension of bacteria with specific O157 antiserum raised in rabbits and absorbed with suspensions of O26 and O55. Autopsy blood cultures in brain-heart infusion broth were incubated at 37°C for 1 week, with subcultures at 1, 3, 5, and 7 days.

**Blood parameters.** Estimation of hemoglobin level, packed cell volume, total and differential leukocyte counts, and serum creatinine levels was done on blood collected before and after infection.

**Histologic examination.** Tissue samples for histologic examination by light microscopy were put in buffered formalin and were embedded in paraffin; sections were stained with hematoxylin and eosin.

**Electron microscopy.** Tissue samples for examination by electron microscopy were fixed in glutaraldehyde, were postfixed in osmium tetroxide, and were processed for ultrastructural study by routine methods. Multiple 1-μm sections from ≥2 blocks of tissue from each site were stained with toluidine blue and were examined. Ultrathin sections were cut on an LKB4 UM4 ultramicrotome (Bromma) with a diamond knife (Eindhoven) and were examined in a Phillips EM201C electron microscope.

**Results**

Seventeen of 22 infected monkeys developed diarrhea, which was watery, self-limiting, and had a maximum duration of 4 days. No monkey had frank, bloody diarrhea, and all stool samples were negative for occult blood. No diarrhea was seen in control samples.

**Blood parameters.** No changes were seen in levels of hemoglobin, platelets, or creatinine in any infected monkeys. An increase in the total leukocyte count and neutrophils was noted in monkeys killed 2 days after infection.

**Bacteriologic examination.** Preinfection cultures of samples from all monkeys and postinfection cultures of control samples were negative for sorbitol nonfermenting E. coli. However, after infection, sorbitol nonfermenting E. coli, which gave a positive agglutination with O157 antiserum, were isolated from stool samples from 17 of the 22 infected monkeys: 16 monkeys had diarrhea and excreted E. coli O157 in the first 4 postinfection days, whereas 1 monkey excreted E. coli O157 without developing diarrhea. Quantitative plate counts showed that 6 of 6 monkeys at postinfection day 6 and 1 of 3 monkeys at postinfection day 9 were excreting >10¹² organisms.

Cultures at autopsy were positive for E. coli O157 in 14 monkeys. Cultures from ≥1 small intestinal sites and all cecum, colon, and rectum cultures were positive in all 14, with monkeys killed at <2 days after infection having positive cultures at all sites. At 2, 3, and 4 days, 1 of 2 monkeys had E. coli O157 present in the terminal ileum, cecum, colon, and rectum. Positive results were obtained for cultures of samples from the terminal ileum, cecum, colon, and rectum of both monkeys killed at 5 and 7 days, 2 of 3 monkeys killed at 9 days, and 0 of 3 monkeys killed at 12 days. All blood cultures were negative for E. coli O157.

At autopsy, 6 of 22 infected monkeys had distension of the terminal small intestine, cecum, and proximal colon. Of the 6 monkeys with abnormal findings at autopsy, 5 were killed ≤5 days and 1 was killed 12 days after infection. No other gross changes were seen.

**Histologic examination.** At light microscopic examination, the most obvious changes were seen in the colon. Rows of bacteria were adherent to the surface epithelium in ≥1 of the 5 segments of colon examined in 15 of 22 infected monkeys, and no adherent bacteria were present in the crypts. Adherent bacteria were more common proximally than distally and were seen within 12 h of infection. They were most common up to postinfection day 4 and then declined, with no adherent bacteria seen at postinfection days 9 and 12.

Mucosal changes (19 of 22 monkeys) were especially striking in the first 5 days after infection. Vacuolization of epithelial cells (14 of 22 monkeys) and epithelial cell tufting and extrusion (10 of 22 monkeys) began 6 h after infection and occurred in all colonic segments of all monkeys on postinfection day 1. These were subsequently more prominent in proximal segments up to 7 days postinfection, after which epithelial cells appeared essentially normal. Widening of intercellular spaces was seen by 12 h after infection, mainly in the ascending and mid-colon, in 11 of 22 monkeys, and persisted for 7 days postinfection. Neutrophil infiltration of the surface epithelium (8 of 22 monkeys) was seen from 6 h to 12 days after infection and was most prominent at 2 days, affecting both proximal and distal colon. The crypts showed mucin depletion and infiltration by neutrophils, with formation of crypt abscesses (12 of 22 monkeys). Crypt abscesses, which were seen from 12 h to 12 days after infection, occurred at all sites but were more prominent in the proximal and mid-colon segments than in the distal colon and rectum. Secondary invasion by a mixed population of bacteria was seen in 7 monkeys in the cecum and ascending and mid-colon from day 5 to day 12 postinfection. The lamina propria showed neutrophil infiltration and mild increase in plasma cells and lymphocytes from 6 h to 12 days. Vascular changes, such as edema, hemorrhage and thrombosis, were not seen. In control monkeys, bacteria adhering to the surface epi-
Electron micrograph of colonic surface epithelium 2 days after infection, showing adherent bacteria forming attaching-effacing lesions, with microvillar effacement and accumulation of actin at the site of attachment at the intercellular junction. There is marked distortion of microvilli adjacent to the lesion. Original magnification, ×42,525.

Figure 1.

Discussion

To our knowledge, we report for the first time a monkey model for EHEC infection, with the formation of A/E lesions in adult monkeys with a normal intestinal microflora. E. coli O157:H7 strain 84-01 colonized the gut, produced diarrhea in 17 of 22 monkeys, and formed the pathognomonic A/E lesions in the cecum and ascending and mid-colon. In earlier studies with other animal models, these lesions were seen variously in the cecum and proximal colon [3], distal colon [12], or the entire colon and distal small intestine [4, 11, 13].

Diarrhea was an early feature of the disease process in this study; it occurred within the first 5 days after infection, and E. coli...
coli O157 was isolated from the diarrheal stools of all diarrheic monkeys but one. Excretion of the organism in the absence of diarrhea, as reported elsewhere for humans [10], also was seen with 1 monkey that excreted E. coli O157 for a week without ever developing diarrhea. The maximum incidence and duration of diarrhea correlated with degenerative surface epithelial changes.

In earlier studies, adherent bacteria have been reported from 18 h after infection in a calf model [11] and up to postinfection day 90 in a chick model [2]. Our study of sequential changes demonstrates that A/E lesions accompanied by surface epithelial changes appear as early as 12 h after infection and represent early features of the disease. Ultrastructural features of the A/E lesion have been described before [11–13] and could account for the loss of normal absorptive function. However, evidence of severe cell damage with gross swelling of cytoplasm and disruption of cell membrane, degenerative swelling of endoplasmic reticulum and mitochondria, and irreversible nuclear injury have not been reported previously. Our findings of severe epithelial damage are indicative of incipient ulcers and correlate well with light microscopic evidence of surface epithelial necrosis described in earlier animal models and in humans [6, 7]. Widened intracellular spaces between surface epithelial cells may indicate active fluid secretion by these cells.

The formation of A/E lesions and the development of striking changes in the surface epithelium were followed by secondary invasion by luminal organisms, which probably causes exacerbation of disease, as seen in the clinical setting. This feature has not been demonstrated in earlier models that used gnotobiotic monkeys. Further studies that use organisms that produce no toxin, either Stx 1 or 2, and that use isogenic deletion mutants for the eae gene in this model are required to characterize the disease process in primates.

References


