Enteropathogenic *Escherichia coli* and Ulcerative Colitis in Cotton-Top Tamarins (*Saguinus oedipus*)

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The cotton-top tamarin (CTT; *Saguinus oedipus*) is an endangered New World primate that develops a highly prevalent idiopathic colitis resembling human ulcerative colitis. This study found that enteropathogenic *Escherichia coli* (EPEC) caused acute colitis in CTTs, which was associated with ulcerative colitis. EPEC clinical isolates revealed localized adherence patterns by HEp-2 assay and were devoid of Shiga-toxin production. Sequencing of the *eae* gene (GenBank accession no. AF319597) revealed 99.2% identity to sequences of human isolates (GenBank AF116899) and corresponded to the ε intimin gene subtype. Detection of intimin sequences by polymerase chain reaction on primary fecal cultures indicated widespread EPEC infection in the CTT colony. Prospective analysis revealed that animals with fecal cultures positive for intimin sequences had a higher frequency of active colitis (75.0% vs. 27.2%; *P* < .005, χ² test) and higher histological scores of colonic inflammation (0.875 vs. 0.455, respectively; *P* < .05, Mann-Whitney rank sum test).

The cotton-top tamarin (CTT; *Saguinus oedipus*) is an endangered primate native to the forests of northern Colombia. A highly prevalent idiopathic colitis resembling human ulcerative colitis has been recognized in CTTs housed at the New England Regional Primate Research Center (NERPRC) and elsewhere since the 1970s. This idiopathic colitis shares similar clinical, endoscopic, and histopathological features with ulcerative colitis in humans and has been widely used as a nonhuman primate model to investigate immunopathogenesis, risk factors, and novel therapeutic interventions for the human condition. CTTs with chronic colitis have a high incidence of colonic carcinoma. Although the etiology of CTT colitis and colonic carcinoma is unknown, several lines of evidence suggest that environmental factors play a role: A prospective study of the epizootiology of colitis and adenocarcinoma showed that chronic mucosal changes were modified by diet [2]. Furthermore, the marked decreased incidence of both colitis and carcinoma of animals raised in isolation from the rest of the colony suggested the existence of an infectious agent as a cofactor in disease [2]. Circumstantial evidence has implicated *Campylobacter jejuni*, corona-like viruses, and *Helicobacter* species as agents that may play a role in producing this disease syndrome [2, 3].

An infectious etiology has also been suggested as a cofactor of ulcerative colitis in humans, and enteroadherent *Escherichia coli* has been implicated in the pathogenesis of this disease. The adhesive properties of *E. coli* isolates from patients with ulcerative colitis differ from those of isolates from healthy control patients, and intimin-positive strains have been identified in some affected persons [4, 5]. Furthermore, treatment of patients with ulcerative colitis by means of probiotic nonpathogenic *E. coli* has an effect similar to that of mesalazine in maintaining remission [6]. Here we describe enteropathogenic *E. coli* (EPEC) infection in CTTs and its association with ulcerative colitis in this species.

**Methods**

**Animals and housing.** CTTs were housed at the NERPRC. All CTTs were housed in pairs or grouped into small extended families consisting of 1 adult breeding male CTT, 1 adult breeding female CTT, and 2–6 siblings <18 months old, as described elsewhere [7]. Average colony census during the study period was ~200 CTTs.

**Bacteriology.** Rectal swabs of clinically affected animals were cultured by standard techniques. Samples were plated on MacConkey, Hektoen, and blood agar at 37°C in 5% CO₂ and on *Campylobacter* agar at 42°C with 5% O₂, 85% N, and 10% CO₂. Plates were evaluated at 24, 48, and 72 h, and individual bacterial colonies were identified by standard biochemical techniques (API Rapid 20E; BioMerieux Vitek). A HEP-2 adherence assay was done as described elsewhere [8]. Bacterial isolates were serotyped at the
Pennsylvania State University. *E. coli* Reference Laboratory (University Park, PA). Shiga-toxin production was assessed by Vero cell cytotoxicity assay, as described elsewhere [9].

**Polymerase chain reaction (PCR) and sequencing.** For characterization of individual bacterial clones, DNA was isolated from single colonies and grown overnight in broth. PCR utilizing primers directed at the intimin (eae), Shiga-like toxin (stx1 and stx2), and hemolysin (hlyA) genes was done as described elsewhere [10]. For detection of bacteria-specific virulence sequences in primary fecal cultures, rectal swabs or colonic tissue were placed in Luria broth (Difco Laboratories) and grown overnight. DNA was then isolated from cell pellets. The intimin gene was amplified from isolates obtained from 2 animals by use of primers SK1 and LP5 [11]. The PCR products were cloned (Invitrogen) and sequenced utilizing an ABI automated sequencer (Perkin-Elmer Cetus) and forward/reverse primers. The bfpA gene was amplified from EPEC isolates, using primers Donne-28 and Donne-29, and sequenced as described elsewhere [12].

**Colonic biopsy specimens and histopathology.** Colonic biopsy specimens were collected, stained with hematoxylin-eosin, and examined in a blinded fashion for acute colitis and evidence of structural alterations associated with chronicity, as described elsewhere [2]. For statistical work, we used a commercial software package (Jandel Scientific). Groups were compared by the contingency or the Fisher’s exact and Mann-Whitney rank sum tests, as appropriate.

**Results**

Five CTTs presented with acute onset of profuse diarrhea. Affected animals became anorexic and inactive and developed clinically recognized dehydration. Laboratory results revealed marked neutrophilic leukocytosis, metabolic acidosis, and hypokalemia. Hyperglycemia and hypoalbuminemia were less frequent. Despite the apparent blood loss in feces, animals did not develop significant anemia. Animals received supportive therapy and were treated with enrofloxacin (2 mg/kg once a day intramuscularly) and lactated Ringer’s solution. Despite the apparent blood loss in feces, animals did not develop significant anemia. Animals received supportive therapy and were treated with enrofloxacin (2 mg/kg once a day intramuscularly) and lactated Ringer’s solution. Despite therapy, 4 of 5 animals died or were euthanized after a disease course of 2–10 days.

Biopsy specimens and/or tissue obtained at necropsy were evaluated from all clinically affected animals, to assess morphological alterations associated with colitis. Findings revealed features diagnostic of an attaching and effacing lesion. Colonic epithelium appeared rounded or irregular, giving the surface a scalloped or cobblestone appearance (figure 1). Bacilli were intimately associated with the apical cytoplasmic membrane, and individual epithelial cells often exfoliated with variable cytoplasmic vacuolization. Surface changes were accompanied by marked crypt cell hyperplasia characterized by loss of goblet cells, cytoplasmatic basophilia, increased mitotic rate, and loss of nuclear polarity. Crypt abscesses were visible in most sections, and neutrophilic infiltrates were often present within the lamina propria. Ultrastructurally, there was effacement of normal microvillus architecture, and adherent bacilli were attached to the apical cytoplasmic membrane with pedestal formation and rearrangement of the underlying cytoskeleton. Morphological alterations were limited to the large intestine, which was diffusely involved in all cases. In contrast, the small intestine was spared and was normal in all animals.

Rectal swabs and fecal samples were obtained from all clinical cases, for bacterial isolation and identification, and were negative for common enteric pathogens of nonhuman primates, including *Salmonella*, *Shigella*, and *Campylobacter* species. Rectal swabs plated on MacConkey agar produced almost pure growth of *E. coli* from all animals. Multiple isolates tested by HEP-2 adherence assay indicated a localized adherence pattern characteristic of EPEC. DNA was isolated from clones from each animal, and the presence of the eae gene was confirmed by PCR amplification of a 384-bp product. A 2.6-kb fragment was amplified from 2 animals and sequenced (GenBank accession no. AF319597). A BLAST similarity search revealed 99.2% identity to sequences obtained from human and rhesus macaque isolates (GenBank AF116899 and AF301015, respectively) that correspond to the eae intimin gene subtype [11, 13]. Similarly, the bfpA gene was detected, by PCR, in EPEC isolates, and sequencing revealed 98.4% identity to bfpA sequences obtained from human EPEC isolates (GenBank AF382948 and AF304484, respectively). PCR performed on isolates was negative for the detection of stx1, stx2, and hlyA sequences. Furthermore, no Shiga-toxin production was demonstrated by the Hela cell cytotoxicity test. Bacterial isolates positive for the eae gene and demonstrating the localized adherence pattern were serotyped and identified as O26:HNM.

To investigate the relationship between EPEC infection and colitis in the CTT colony, we did a prospective analysis to determine the incidence of EPEC infection and its relationship to ulcerative colitis. Paired fecal and colonic biopsy specimens were obtained from colony animals (n = 38) 2 weeks after resolution of the last clinical case and were evaluated for the presence of intimin sequences by PCR and of inflammatory infiltrates by histopathology. Of 38 animals, 16 (42%) were PCR positive for intimin sequences in primary stool cultures. PCR performed on fecal cultures was negative for the detection of stx1, stx2, and hlyA sequences.

Twelve of the 16 animals positive, by PCR, for intimin sequences had histological evidence of active colitis, compared with 6 (27.2%) of 22 that were PCR negative (P < .005, χ² test). Mean scores for active colitis were significantly higher for animals with fecal cultures positive for intimin sequences, compared with those for animals with negative cultures (mean, 0.875 vs. 0.455, respectively; P < .05, Mann-Whitney rank sum test). No significant differences between animals with positive and those with negative fecal cultures were noted for chronicity scores (mean, 0.875 vs. 0.864, respectively) or age (8.5 and 9.5 years, respectively). An attaching and effacing lesion was identified morphologically in only 1 of the 12 animals with active colitis that had fecal cultures positive for intimin sequences. In
this animal, the lesion was present multifocally in 1 of 3 colonic sections examined. In the remaining intimin-positive animals, inflammatory changes did not differ from those typically attributed to idiopathic ulcerative colitis in CTTs.

The colony was retested for the presence of EPEC 7 months after the first survey. Of 35 animals tested, intimin sequences were detected by PCR on DNA isolated from primary fecal cultures from 12 (34.3%) animals. Despite the absence of severe acute colitis, the incidence of infection was not statistically different from that 7 months earlier.
Discussion

Here we describe EPEC infection as a cause of severe acute colitis in CTTs. The clinical disease was characterized by profuse and sometimes hemorrhagic diarrhea and was often accompanied by neutrophilic leukocytosis, metabolic acidosis, and hypokalemia. Animals with acute disease had morphological findings characteristic of EPEC infection in other species, including the diagnostic attaching and effacing lesion. Definitive diagnosis was obtained by histological evaluation and was supported through bacteriologic culture and by use of PCR and the HEp-2 adhesion assay. Sequencing of a 2.6-kb fragment of the intimin gene revealed 99.2% identity to sequences obtained from human EPEC isolates and corresponded to the ϵ subtype. This subtype was previously associated with enterohemorrhagic E. coli (EHEC) isolates obtained from cattle and humans and from rhesus macaques with AIDS [11, 13]. There was no evidence that isolates from CTTs produced Shiga toxin, as determined by cell cytotoxicity assays or PCR, nor were histomorphological findings at death compatible with hemolytic uremic syndrome. PCR also identified bfpA sequences consistent with the observed localized adherence phenotype.

Molecular techniques showed widespread EPEC infection in the rest of the CTT colony. Routine cultures for common bacterial pathogens in nonhuman primates will likely underestimate the true incidence of EPEC infection, and specialized techniques must be pursued for definitive diagnosis. E. coli is the predominant facultative anaerobe found within the colon, and most clinical laboratories do not distinguish between species of lactose fermenters or diarrheagenic from nondiarrheagenic E. coli. Furthermore, differentiation requires specialized techniques such as PCR and adhesion assays that may not be readily available in most clinical laboratories. Although colonic biopsy was reliable for the identification of animals with clinical disease, PCR performed on DNA isolated from primary fecal cultures was required to identify enzootic infection in the CTT colony.

As in humans with ulcerative colitis, the etiology of colitis in CTTs is unknown, and a variety of factors have been implicated in producing the full clinical syndrome, including diet, environmental stress, and genetic influences [7, 14]. Wild CTTs in their natural environment have a markedly decreased incidence of colitis, epithelial dysplasia, and overt carcinoma, compared with captive animals, indicating that environmental factors initiate or promote disease development [14]. It has also been suggested that infectious agents may play a role in the clinical manifestation of disease. Corona-like viruses and Campylobacter and Helicobacter species have been suggested as potential pathogens that may initiate or influence disease course [2, 3].

EPEC infection in the CTT colony was a common enzootic infection, and the incidence was essentially unchanged over a 7-month observation period. Although animals with clinically unrecognized EPEC infection had a higher incidence of colitis and higher histological scores of colitic activity, whether EPEC infection plays an initiating or an etiologic role in producing colitis remains undetermined. For a variety of reasons, animals with preexisting colitis have altered mucosal defenses and may be prone to colonization by potentially pathogenic organisms. Such changes may include alterations in lipid metabolism, mucosal architecture, microbial flora, epithelial maturity, and cell surface receptor expression. Of particular interest may be changes in phospholipid turnover found in inflamed mucosa, leading to increased phosphatidylethanolamine levels. Expression of phosphatidylethanolamine was previously correlated with binding of EHEC and EPEC strains [15].

Although animals with preexisting colitis may be more readily colonized by potential pathogens, EPEC may potentiate chronic inflammatory changes through alterations in mucosal permeability and activation of proinflammatory signaling pathways. EPEC infection of CTTs may provide an experimental system with which to investigate the role of adherent bacteria in the pathogenesis of ulcerative colitis in humans and an opportunity to examine whether infection potentiates mucosal injury.

References

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