CONCISE COMMUNICATION

Ferrets as a Model System for Renal Disease Secondary to Intestinal Infection with Escherichia coli O157:H7 and Other Shiga Toxin–Producing E. coli

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Ferrets were evaluated as a possible small animal model for the development of colitis and/or signs of the hemolytic uremic syndrome after oral infection with Escherichia coli O157:H7 or other Shiga toxin–producing E. coli (STEC). Ferrets treated with streptomycin (Stm) had higher counts of E. coli O157:H7 strain 86-24 Stm-resistant (Stm-) or O91:H21 strain B2F1 Stm- in their stools than non–Stm-treated animals. None of the animals displayed evidence of colitis, but Stm-treated animals fed strain 86-24 Stm- exhibited weight loss significantly greater than that exhibited by ferrets fed an isogenic mutant negative for the adhesin intimin. Moreover, 11 (23%) of the 47 Stm-treated ferrets inoculated with 86-24 Stm- or B2F1 Stm- developed hematuria and/or histological damage to glomeruli or thrombocytopenia, compared with 0 of 14 uninfected control animals receiving Stm in water. Thus, the ferret may serve as a model for renal disease secondary to intestinal infection with STEC.

Escherichia coli O157:H7 is the most common infectious cause of bloody diarrhea in the United States and is responsible for an estimated 74,000 illnesses per year [1]. Indeed, infection with E. coli O157:H7 frequently leads to hemorrhagic colitis (HC), a term used to describe the bloody stools, inflammation, and colon damage exhibited by many of the patients infected with this organism [2]. The potentially serious nature of E. coli O157:H7 infection is illustrated by the fact that ~6% of those infected, particularly children, develop a sequela called the hemolytic uremic syndrome (HUS) [3], which is characterized by microangiopathic hemolytic anemia, thrombocytopenia, renal failure, central nervous system involvement, and, in 3%–5% of children, death [4]. A second possible sequela of E. coli O157:H7 infection, thrombotic thrombocytopenic purpura, presents in adults and is similar to HUS but with more frequent occurrence of neurologic symptoms than of renal failure [4].

Although E. coli O157:H7 infections account for many cases of HC and HUS, other serotypes of E. coli that make ≥1 types of Shiga toxin (Stx; collectively called Shiga toxin–producing E. coli, or STEC) are estimated to contribute another 36,000 illnesses annually [1]. E. coli O157:H7 belongs to a subset of STEC termed enterohemorrhagic E. coli, which not only make Stx but also use the adhesin intimin to attach to the bowel and evoke an attach and efface (A/E) lesion at the site of the bacteria-enteroocyte interface [5].

The pathogenic process by which E. coli O157:H7 and other STEC evoke bloody diarrhea, HUS, and thrombotic thrombocytopenic purpura remains incompletely understood, in part due to the lack of effective animal models. Specifically, no small animal model has been described in which Stx-mediated HC with renal glomerular disease follows enteral inoculation with a human STEC strain. The domestic ferret is presently the only animal model that develops diarrhea after enteral inoculation with Campylobacter jejuni [6]. For this reason, we chose to explore the possibility that ferrets might serve as an effective model for one or more aspects of human STEC-mediated disease.

Materials and Methods

Bacteria. Three spontaneous streptomycin (Stm)–resistant (Stm-) STEC strains were used: O91:H21 strain B2F1 Stm- (a natu-
raly intimin-negative STEC), O157:H7 strain 86-24 Stm\(^{r}\), and strain 86-24eae\(\Delta 10\) Stm\(^{r}\), an isogenic intimin mutant of strain 86-24 Stm\(^{r}\) [7]. Strains 86-24 and B2F1 (provided by P. Tarr, Children’s Hospital and Medical Center, Seattle, and M. Karmali, Laboratory for Foodborne Zoonoses, Health Canada, Guelp, Canada, respectively) were originally isolated from patients with STEC illnesses.

**Animals.** Six-week-old descended, *Campylobacter*-free female ferrets (*Mustela putorius furo*) were obtained from Marshall Farms.

**Oral infection model.** For Stm treatment, ferrets were provided with water containing 1–5 g Stm/L 24 h before inoculation and at all times thereafter. All food and water were removed from cages 4 h before inoculation. The bacterial inoculum was prepared by centrifugation of 10-mL overnight cultures and resuspension of the bacteria in 2 mL of milk or PBS, pH 7.4. In initial studies, ferrets were scuffed and were allowed to drink the inocula from plastic transfer pipettes. In subsequent experiments, the bacterial suspension was delivered directly into the stomach via a 5 French pediatric feeding tube. Animals received a total inoculum of 10\(^{10}\)–10\(^{11}\) cfu in 1 dose or in 2 doses administered 4–5 h apart. Following inoculation, food and water were returned to the cages.

Ferrets were monitored daily, and animal weights were obtained every 4–7 days in initial studies and every day in later experiments. Stool and urine were also examined daily. Intestinal colonization was determined by fecal collection. Feces were diluted and plated onto sorbitol MacConkey agar supplemented with 200 µg/mL Stm to determine the number of colony-forming units per gram of feces. Agglutination with commercially available anti-O157 or anti-H21 sera (provided by N. Stockbine, Centers for Disease Control and Prevention, Atlanta) was also used to type bacteria when necessary.

**Hematology and histology.** At necropsy, blood was obtained for culture and laboratory analyses that included complete blood cell count, blood urea nitrogen, creatinine, and red blood cell morphology. Kidneys and intestinal samples were also collected for histologic evaluation. Formaldehyde-fixed intestinal samples were stained with hematoxylin-eosin (HE) and occasionally were assessed for A/E lesion formation by indirect immunohistochemistry with anti-O157:H7 antibody. Kidney sections were stained with HE and phosphotungstic acid-hematoxylin.

**Results**

**Evaluation of the requirement for Stm treatment in the colonization of ferrets with various STEC strains.** We previously reported that high-level colonization of mice orally infected with STEC requires pretreatment of the animals with Stm [8]. To assess whether Stm treatment would provide greater STEC colonization of ferrets, 2 small-scale experiments were done. In the first study, 2 of 4 ferrets were treated with Stm before inoculation with strain 86-24 Stm\(^{r}\). In the absence of Stm treatment, ferrets were colonized with 86-24 Stm\(^{r}\) at low to moderate levels (range of geometric mean [GM] counts, 1.5 × 10\(^{2}\) to 2.5 × 10\(^{4}\) cfu/g of feces) from postinoculation days 1–6, but the levels were below the limit of detection (<10\(^{2}\) cfu/g feces) by postinoculation day 8. The Stm-treated ferrets were colonized at high levels (GM, ≥1.3 × 10\(^{5}\) cfu/g of feces) with strain 86-24 Stm\(^{r}\) throughout the 8-day experiment.

In the second study, 2 untreated and 2 Stm-treated ferrets were inoculated with the highly virulent, naturally intimin-negative STEC strain B2F1 Stm\(^{r}\) [8]. After postinoculation day 1, untreated ferrets shed little to no detectable B2F1 Stm\(^{r}\). By contrast, moderate to high levels (GM, 8.6 × 10\(^{4}\) to 2.7 × 10\(^{8}\) cfu/g of feces) of B2F1 Stm\(^{r}\) were detected in Stm-treated ferrets throughout the 12-day experiment, and 1 animal in this group developed macroscopic hematuria that persisted for 8 days. In both experiments, Stm treatment appeared to markedly enhance the level and duration of STEC colonization.

**Examination of the role of intimin in ferrets colonized with E. coli O157:H7.** To address what role intimin played in the colonization of ferrets by *E. coli* O157:H7, 2 experiments were done. In the first study, groups of 3 Stm-treated ferrets were inoculated with strain 86-24 Stm\(^{r}\) or with the isogenic intimin mutant strain 86-24eae\(\Delta 10\) Stm\(^{r}\). On day 1 after inoculation, the 2 groups had approximately equal levels of colonization; however, on post-infection days 2–9, ferrets inoculated with strain 86-24 Stm\(^{r}\) shed more (0.5–2 logs higher) bacteria than animals colonized with the intimin-negative isogenic mutant (GM range, 6.5 × 10\(^{5}\) to 1.2 × 10\(^{8}\) vs. 2.6 × 10\(^{7}\) to 1.1 × 10\(^{8}\) cfu/g of feces). These consistent differences were not statistically significant, probably due to the small sample sizes. One 86-24 Stm\(^{r}\)–inoculated ferret was killed on postinoculation day 6 after the animal developed macroscopic hematuria (subsequent histologic examination revealed evidence of renal papillary necrosis in this animal). Nevertheless, at postinoculation day 6, the last day with 3 animals per group, ferrets inoculated with 86-24 Stm\(^{r}\) showed a statistically significant weight loss (~11% net), compared with a net weight gain (+17%) in 86-24eae\(\Delta 10\) Stm\(^{r}\)–inoculated ferrets (\(P = .05\), 1-sided Wilcoxon 2-sample test).

In the second study, we assessed whether intimin could alter colonization levels in the presence of competing normal flora in non–Stm-treated animals. Six untreated ferrets were inoculated with strain 86-24 Stm\(^{r}\) (\(n = 4\)) or strain 86-24eae\(\Delta 10\) Stm\(^{r}\) (\(n = 2\)) and were monitored for 6 days. All ferrets showed colonization (3.5 × 10\(^{5}\) to 4.4 × 10\(^{6}\) cfu/g of feces) on day 1 after inoculation; however, colonization levels in animals inoculated with the intimin mutant strain were below the limit of detection by postinoculation day 2 and generally remained so thereafter. All untreated ferrets inoculated with the intimin-positive strain 86-24 Stm\(^{r}\) maintained low to moderate levels of colonization (GM, 4.9–7.8 × 10\(^{5}\) cfu/g feces) until postinoculation day 3 before gradually declining to <10\(^{5}\) cfu/g of feces by day 6, except for 1 animal that remained moderately colonized (1.7 × 10\(^{5}\) to 8 × 10\(^{6}\) cfu/g of feces) throughout. In a repeat of this experiment (\(n = 3\) ferrets/group), the general trend remained that intimin enhanced O157:H7 colonization; however, comparable net weight gains (11%–27%) occurred among ferret groups. Together, these experiments indicate that the presence of intimin provides an ad
Figure 1. Characteristic histologic specimens of kidneys from ferrets inoculated with Shiga toxin–producing *Escherichia coli*. A, Non–streptomycin (Stm)–treated, uninfected 9-week-old control ferret glomerulus stained with hematoxylin-eosin (HE). B, HE-stained glomerulus from an Stm-treated, B2F1 Stm-resistant (Stm r)–infected ferret with gross hematuria 28 days after inoculation. HE-stained (C) and phosphotungstic acid–hematoxylin–stained (D) glomeruli from another Stm-treated, B2F1 Stm r–infected ferret with no evidence of hematuria 10 days after inoculation. D, Dark blue stained areas within the glomerular capillaries are probably fibrin clots (arrow). Original magnification, ×100 for all panels.
vantage in O157:H7 colonization of the ferret and that this advantage in Stm-treated animals translates to increased illness in the context of weight loss.

Analysis of intestinal and kidney sections from STEC-infected ferrets. Despite the occasional appearance of loose and/or watery stools on postinoculation days 1–2 in both Stm-treated 86-24 Stm'–infected and B2F1 Stm'–infected ferrets, light microscopy did not reveal evidence of colitis or, in the case of O157:H7 infection, A/E lesions. Examination of kidney sections indicated that only Stm-treated ferrets infected with 86-24 Stm' or B2F1 Stm' developed renal damage. Unlike the glomerulus of an uninfected control ferret (figure 1A) or an Stm-treated animal (not shown), the glomerulus of a B2F1 Stm'–infected ferret with macroscopic hematuria appeared grossly enlarged, with pale nuclei and narrowed capillary lumens (figure 1B). Evidence of kidney damage (pyknotic nuclei, fibrillar cytoplasm, narrowed capillary lumens, and probable fibrin clots within the glomerular capillaries) was also apparent in ferrets without macroscopic hematuria (figure 1C and 1D).

Summary of renal and hematologic findings. The results obtained from Stm-treated ferrets infected with 86-24 Stm' or B2F1 Stm' are summarized in table 1. Most striking was the finding that 23% of the STEC-inoculated animals showed ≥1 significant sign of HUS (i.e., renal glomerular disease and/or thrombocytopenia). Renal damage was characterized predominantly by small patches of affected glomeruli, with occasional patches of cortical necrosis. No acute tubular necrosis was evident in infected animals. The renal glomerular damage revealed by light microscopy was similar among all affected ferrets and was consistent with that seen in cases of human HUS [9–11]. Hematologic analysis revealed no gross abnormalities between STEC-infected ferrets and uninfected controls; however, 2 Stm-treated STEC-infected ferrets with renal damage also exhibited thrombocytopenia at the time of necropsy (platelets ≤150 K/μL vs. 200–1000 K/μL in controls).

Discussion

Three major conclusions can be drawn from these studies. First, Stm treatment of ferrets enhanced intestinal colonization by all STEC strains tested and was essential for colonization of animals by B2F1 Stm'. Second, STEC colonization of non-Stm-treated and Stm-treated ferrets was enhanced by the presence of the bacterial adhesin intimin. In addition, Stm-treated ferrets colonized with intimin-positive strain 86-24 Stm' exhibited more illness, as indicated by weight loss, than those animals infected with an isogenic intimin mutant. Third, and most important, renal histologic damage consistent with human cases of STEC-mediated HUS was seen at a significant rate in these studies.

Several animal models have been described for studying the pathogenesis of E. coli O157:H7– or STEC-mediated disease [12, 13]; however, no model entirely reflects the pathogenesis of human infection, which includes the development of diarrhea and HC, with at least occasional HUS-related renal damage, after oral inoculation of a human STEC strain. The Stm-treated ferret model described here is the first model in which animals administered STEC orally or gastrically maintained a localized infection but showed glomerular lesions akin to those documented in patients with HUS [9–11]. Indeed, 23% of Stm-treated ferrets inoculated with STEC strains developed histologic evidence of renal disease, with glomerular damage evident in 9 animals and papillary necrosis apparent in another ferret. Furthermore, preliminary electron microscopic examination of kidney samples from these animals indicated an even higher percentage of animals with renal damage than was detected by light microscopy alone (data not shown). In addition to these histologic findings, the occasional development of hematemia in STEC-infected ferrets and the absence of detectable bacteremia are both consistent with some of the clinical features reported in human cases of HUS [4, 14, 15]. Last, although only 2 animals with evidence of renal disease developed thrombocytopenia, it is possible that subtle or intermittent hematologic abnormalities were missed, since blood was collected only at the time of necropsy.

The likelihood that Stx2 or Stx2d from 86-24 Stm' or B2F1 Stm', respectively, was responsible, directly or indirectly, for the glomerular damage noted in some Stm-treated STEC-infected ferrets is based on epidemiologic data [4] and on evidence from the Stm-treated, orally infected mouse model [8]. However, further studies will be required to prove this hypothesis. Specifically, we will need to assess whether anti-Stx2 antibodies protect ferrets from glomerular damage and whether toxin directly

Table 1. Summary of renal pathology in streptomycin (Stm)–treated ferrets infected with Shiga toxin–producing Escherichia coli.

<table>
<thead>
<tr>
<th>Infecting strain</th>
<th>No. with gross hematuria/ total no. (%)</th>
<th>No. with renal damage/total no. (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Glomerular</td>
<td>Papillary necrosis</td>
</tr>
<tr>
<td>86-24 Stm'</td>
<td>4/35 (11)</td>
<td>7/35 (20)</td>
</tr>
<tr>
<td>B2F1 Stm'</td>
<td>1/12 (8)</td>
<td>2/12 (17)</td>
</tr>
<tr>
<td>Uninfected</td>
<td>0/14 (0)</td>
<td>0/14 (0)</td>
</tr>
</tbody>
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NOTE. Stm', Stm-resistant.

a Two of these animals displayed macroscopic hematuria.

b This animal displayed macroscopic hematuria.

c One of these animals displayed macroscopic hematuria.
injected into the ferret mimics the renal injuries observed in this study. If such experiments prove the critical role of Stx in this model, then the ferret may become the animal model of choice to evaluate toxin-blocking treatments, such as antitoxin or receptor analogues.

Acknowledgment

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References