Renal Injury Is a Consistent Finding in Dutch Belted Rabbits Experimentally Infected with Enterohemorrhagic *Escherichia coli*

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Enterohemorrhagic *Escherichia coli* (EHEC) produces Shiga toxin (Stx) and causes renal disease in humans. Dutch Belted (DB) rabbits naturally infected with EHEC O153 develop hemolytic-uremic syndrome–like disease. The aims of this study were to experimentally reproduce O153-induced renal disease in DB rabbits and investigate bacterial and host factors involved in pathogenesis. The pathogenicity of *E. coli* O157:H7 was also investigated in rabbits. The stx1AB region of O153 was sequenced. By use of liquid chromatography–tandem mass spectrometry, we identified homologs of the Stx receptor, globotriaosylceramide (Gb3), in rabbit kidney extracts. Infected rabbits developed clinical signs and intestinal and kidney lesions. Renal pathological changes consisted of intimal swelling, perivascular edema, erythrocyte fragmentation, capillary thickening, luminal constriction, leukocytic infiltration, mesangial deposits, and changes in Bowman’s capsule and space. Sequence analysis of a ≈7-kb region of the O153 chromosome indicated homology to the Stx1-producing bacteriophage H19B. Our findings indicate that DB rabbits are suitable for the study of the renal manifestations of EHEC infection in humans.

*Escherichia coli* O157:H7 is the prototype enterohemorrhagic strain of *E. coli* (EHEC) and causes a broad spectrum of human disease, including asymptomatic infection, diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) [1]. HUS is characterized by microangiopathic hemolytic anemia and thrombocytopenia, is a leading cause of acute renal failure in children, and develops in 10%–15% of infected patients [2, 3]. Approximately 3%–5% of patients die during the acute phase, and 25% of survivors experience long-term renal sequela [4, 5]. There is no specific treatment, and supportive care does not always give satisfactory results [6, 7].

Shiga toxins (Stxs) are considered to be major virulence factors involved in the pathogenesis of EHEC-induced disease. EHEC produce Stx1 and Stx2 (or variants) alone or in combination. Stx2-producing *E. coli* strains have the highest risk of causing HUS [8, 9]. *E. coli* O157:H7, the serotype most commonly associated with human infection, typically carry the gene encoding Stx2, and approximately two-thirds of these strains have the gene encoding Stx1 [10]. In the proposed model of pathogenesis, Stx within the intestinal tract crosses the epithelial barrier, enters the bloodstream, and targets tissues expressing the receptor globotriaosylceramide (Gb3) [11, 12].

We previously described an outbreak of hemorrhagic diarrhea and HUS-like disease in Dutch Belted (DB) rabbits naturally infected with EHEC O153 [13]. Rabbits are a newly recognized reservoir host of EHEC [14]. These findings prompted studies designed to experi-
mentally reproduce and characterize EHEC-induced renal disease in this naturally susceptible host.

MATERIALS AND METHODS

Strains, fluorescent actin staining (FAS) assay, and culture conditions. EHEC O153 (polymerase chain reaction [PCR] positive for stx1 and eae and negative for stx2) was isolated from DB rabbits with HUS-like disease [13]. This is a sorbitol-fermenting strain. EHEC O157:H7 (CDC EDL 933; ATCC 43895) (PCR positive for stx1, stx2, and eae) is a sorbitol-nonfermenting strain isolated from raw hamburger meat that was implicated in a hemorrhagic colitis outbreak in humans. The FAS assay for EHEC O153 was performed on Caco-2 cells [15].

A single colony of EHEC was inoculated into 10 mL of Luria-Bertani (LB) broth and grown overnight at 37°C; 5 mL of the overnight broth culture was inoculated into 200 mL of prewarmed LB broth and incubated at 37°C for 2 h to reach logarithmic growth phase (OD660, 0.2). The broth culture was centrifuged (10,400 g for 20 min), and E. coli were resuspended in 40 mL of sterile PBS. Serial 10-fold dilutions of E. coli were plated and incubated overnight at 37°C to calculate the number of viable bacteria.

Rabbits. The possibility of age-related susceptibility to EHEC-induced disease was investigated in 2 separate experiments; in one, 16 8-week-old DB rabbits were used, and, in the second, 14 5-week-old DB rabbits were used. Rabbits were housed in Association for Assessment and Accreditation of Laboratory Animal Care–approved facilities. The Massachusetts Institute of Technology Animal Care Committee approved these studies.

Experimental design. Fecal samples were collected from the rabbits before inoculation and were cultured, and isolates were analyzed by PCR for the presence of eae, stx1, and stx2 [13]. Rabbits were fasted overnight, and water was removed at least 2 h before inoculation. All rabbits were given food and water ad libitum after inoculation and were sedated for orogastric intubation.

For experiment 1, rabbits were divided into 2 experimental groups (group 1, n = 6 [2 males and 4 females]; group 2, n = 5 [2 males and 3 females]); and a control group (n = 5 [2 males and 3 females]). Group 1 rabbits were inoculated with O153 (5 × 10^6 cfu), and group 2 rabbits were inoculated with O157:H7 (9 × 10^6 cfu). For experiment 2, rabbits were divided into experimental (n = 9 [all females]) and control (n = 5 [all females]) groups and were infected with O153 (5 × 10^6 cfu). Experimental and control rabbits received 10 mL of 10% sterile sodium bicarbonate orally, to neutralize gastric acidity [16]. Each rabbit in the experimental group was orally inoculated with EHEC resuspended in sterile PBS [16–18]. Control rabbits received sterile PBS.

Fecal EHEC quantification. Fecal pellets were collected for culture, and shedding was determined quantitatively. Serial dilutions of fecal suspensions were plated on selective media and incubated overnight at 37°C to calculate the number of viable bacteria per gram of feces. Bacterial colonies were selected from plates for DNA extraction and confirmation of the inoculum by PCR of stx1 [13].

Clinical assessment. Rabbits were weighed and monitored daily for clinical signs. Blood samples for complete blood count and biochemical analyses were obtained before inoculation and preceding euthanasia. Hematologic values were compared with clinical reference data available for this rabbit breed. Rabbits were euthanized on the basis of the severity of clinical signs and at selected time points during a period of 10 and 17 days after inoculation.

Processing for light microscopy and transmission electron microscopy (TEM). Complete necropsies were performed. For light microscopy, selected tissue specimens were fixed in 10%
Figure 2. Daily postinoculation (pi) change in percentage body weight after inoculation with enterohemorrhagic Escherichia coli. A and B, Experiment 1: rabbits infected at 8 weeks of age with O153 (mean, −18.1%; SE, 4.7%; P < .025 [A]) or O157:H7 (mean, −4.6%; SE, 1.6%; P < .025 [B]). C, Experiment 2: rabbits infected at 5 weeks of age with O153 (mean, −14.7%; SE, 4.2%; P < .0001). Asterisks indicate the rabbits with the most-severe clinical signs (acute disease).

neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 2 or 4 μm, and stained with hematoxylin-eosin (H-E). Kidney sections were also stained with Carstairs’ stain for fibrin.

For TEM, cecum and kidney samples from an acutely affected rabbit and a control rabbit were fixed in 2.5% gluteraldehyde, 3% paraformaldehyde, and 5% sucrose in 0.1 mol/L sodium cacodylate buffer, postfixed in 1% OsO4 in veronal-acetate buffer, stained in block overnight with 0.5% uranyl acetate in veronal-acetate buffer, and then dehydrated and embedded in epon-812 resin. Sections were cut on a Leica ultra cut UCT at 70 nm, stained with 2.0% uranyl acetate followed by 0.1% lead citrate, and examined using a Philips EM 410 electron microscope.

Histopathological assessment and peptic nucleic acid–fluorescent in situ hybridization (PNA-FISH). H-E–stained intestinal sections were assessed for mucosal changes and graded for infiltration of heterophils in the lamina propria, edema, and vascular changes [16]. E. coli Flu PNA probe was used to detect adherent E. coli [13].

For renal lesions, we developed a scoring system based on diagnostic criteria for HUS in humans [19]. It incorporates 6 categories of glomerular lesions, 3 categories of arterioles/arteries, and morphologic assessment of erythrocyte fragmentation. Each category was graded on a 0–4 scale based on defined criteria, by a pathologist blinded to the treatment group of the samples.

Statistics. Analyses were performed using Minitab statistical software (release 13; Minitab). A 2-sample t test was used to analyze body weight changes. A Mann-Whitney test was used to analyze the pathology scores. Tests were considered significant at P < .05.

Sequencing. O153 chromosomal DNA (7333 bp) encompassing the stx1AB region from the Q gene through phage lysis genes S, R, and Rz was amplified by PCR as follows. The region downstream of stx1B was amplified by long-range PCR, using primers designed from the known sequence of stx1B from phage H19B (5′-TGCCTGTCTAAATGAGGGGGATTCAGCAGGGAAGT-3′) and from a conserved region of Rz from phage 933W (5′-CGCGCTGCGCTTTGTAGGTGAGCGCGTTGTCAC-3′). The resulting PCR product was cloned into the PCR cloning vector pGEM-T, and both strands were sequenced. This region was found to be highly homologous to the Stx1-encoding bacteriophage f4795 from E. coli O84:H4 strain 4795/97 (GenBank accession number AJ556162). Primers were designed from the known sequence of phage f4795 and used to amplify the region from the upstream Q gene through stx1B. Both strands of the amplicons were sequenced.

Extraction of glycolipids and Gb3 analyses. Kidney tissue from a DB and a New Zealand White (NZW) rabbit was separated into cortex and medulla. The tissue was suspended in water and homogenized. Glycolipid extraction and purification was performed as described elsewhere [20]. The collected fractions were dried and dissolved in ethanol. Gb3 homologs in the samples were identified by comparison with a known porcine standard (Matreya), using 2 methods: thin-layer chromatography (TLC) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). For TLC, an aliquot of each tissue extract dissolved in ethanol was separated using chloroform/methanol/water (60:40:9 vol/vol) and detected using 10% sulfuric acid in methanol [21]. For LC-MS/MS, we used an Agilent 1100 binary pumping system with a C18 column interfaced with an Agilent XCT MSD Trap mass spectrometer.
RESULTS

**FAS assay.** EHEC O153 caused accumulation of cytoskeletal actin into pedestals beneath cell-associated bacteria (figure 1B).

**Clinical findings.** Before inoculation, all rabbits were negative by PCR for *E. coli* carrying the *eae* and/or *stx* genes. Control rabbits had no clinical signs, whereas 100% of the rabbits inoculated with O153 in both experiments exhibited diarrhea, lethargy, inappetence/anorexia, dehydration, and weight loss by day 3. In experiment 1, 2 (33%) of 6 O153-infected rabbits developed acute disease prompting euthanasia on day 6. Infected rabbits lost significant weight by day 6 (mean, \(-18.1\); SE, 4.7%; \(P < .025\)) (figure 2A).

Eighty percent of the rabbits inoculated with O157:H7 exhibited diarrhea by day 2. Two (40%) of five O157-infected rabbits developed acute disease prompting euthanasia on days 3 and 6. Infected rabbits lost significant weight by day 6 (mean, \(-4.6\); SE, 1.6%; \(P < .02\)) (figure 2B).

Rabbit 1 (experiment 2) developed hemorrhagic diarrhea and was euthanized on day 3. Two other rabbits developed severe clinical signs prompting euthanasia on day 7. These 3 rabbits (33%) developed acute disease with marked weight loss. Infected rabbits lost significant weight by day 6 (mean, \(-14.7\); SE, 4.2%; \(P < .0001\)), including rabbit 7, which continued to be clinically affected (figure 2C).

**Clinical pathological assessment.** In experiment 1, 5 of 6 rabbits infected with O153 developed low hematocrit values, ranging from 24%–37% (normal range, 38%–45.5%). Rabbit 3 also exhibited a white blood cell (WBC) count of 9.94 \(\times 10^3\) cells/\(\mu\)L (normal range, \(6.3 \times 10^3\)–13 \(\times 10^3\) cells/\(\mu\)L), relative heterophilia (85%; normal range, 30%–50%), thrombocytopenia (176 \(\times 10^3\) platelets/\(\mu\)L; normal range, 221 \(\times 10^3\)–463 \(\times 10^3\) platelets/\(\mu\)L), hypoproteinemia (4.0 g/dL; normal range, 4.8–9.5 g/dL), and hyperfibrinogenemia (410 mg/dL; normal range, 100–400 mg/dL). Rabbit 5 with anemia (24%) developed burr cells, a WBC count of 11.90 \(\times 10^3\) cells/\(\mu\)L with relative heterophilia (87%), an elevated blood urea nitrogen (BUN) level (27–28 mg/dL) and/or hypoproteinemia (4.3 g/dL), hypoalbuminemia (2.5 g/dL; normal range, 2.7–3.7 g/dL), and hyperfibrinogenemia (720 mg/dL). Rabbits (3/5) infected with O157:H7 developed low hematocrit values, ranging from 31% to 37%. Rabbits 2 and 3 developed thrombocytopenia (76 \(\times 10^3\) platelets/\(\mu\)L) and hyperfibrinogenemia (1160 mg/dL), respectively.

In experiment 2, the 3 rabbits with acute disease developed mild elevations in BUN (27–28 mg/dL) and/or hypoproteinemia.
EHEC-Induced Renal Injury in Rabbits

Figure 4. Renal histopathological evaluation. A, Experiment 1: rabbits infected with O153. B, Experiment 1: rabbits infected with O157:H7. C, Experiment 2: rabbits infected with O153. P values for comparison between control and infected rabbits in graphs A, B, and C, respectively, are as follows: capillary thickening (CT), \( P < .01 \), \( P < .01 \), and \( P < .003 \); luminal constriction (LC), \( P < .01 \), \( P < .01 \), and \( P < .003 \); fibrin thrombi (FT), \( P = .09 \), \( P = 1.0 \), and \( P = .28 \); glomerular white blood cells (mainly heterophils) (WBC), \( P < .01 \), \( P < .01 \), and \( P < .003 \); mesangial deposits (M), \( P < .01 \), \( P < .01 \), and \( P < .002 \); Bowman’s capsule and space (BCS), \( P = .09 \), \( P = .44 \), and \( P < .007 \); intimal swelling (IS), \( P < .01 \), \( P < .01 \), and \( P < .002 \); mural degeneration (MD), \( P = .24 \), \( P = .44 \), and \( P = .05 \); perivascular edema (PE), \( P < .01 \), \( P < .01 \), and \( P < .002 \); and red blood cell fragmentation (RBCf), \( P < .02 \), \( P < .02 \), and \( P < .002 \).

EHEC recovery from feces. O153 shedding during the first week was variable among infected rabbits (\( 10^5 \)–\( 10^9 \) cfu/g of feces); however, rabbits with acute disease shed the highest levels (\( \geq 10^7 \)–\( 10^9 \) cfu/g of feces) every day until euthanasia. Rabbits inoculated with O157:H7 shed \( 10^6 \)–\( 10^7 \) cfu/g of feces on day 1 and \( 10^5 \) cfu/g of feces on day 2; however, from day 3 to day 7, the number of colony-forming units per gram of feces decreased to \( 10^2 \)–\( 10^5 \). PCR analyses detected \( stx1 \) in isolates from EHEC-infected rabbits.

Necropsy findings. In 3 (50%) of 6 O153-infected and 1 (20%) of 5 O157-infected rabbits from experiment 1, serosal surfaces of the cecum and/or proximal colon had petechial or echymotic hemorrhages and were edematous and thickened. Rabbit 5 of the O153-infected group had pale kidneys and lacked perirenal adipose tissue. In experiment 2, a necropsy of rabbit 1 on day 3 revealed an intussusception of the jejunum into the proximal ileum, as well as intestinal hemorrhages.

Histopathological assessment. Compared with control rabbits (figure 3A), infected rabbits from experiment 1 and acutely affected rabbits from experiment 2 developed edema of the cecum (experiment 1 [groups 1 and 2, respectively], \( P < .04 \) and \( P = .10 \); experiment 2, \( P < .03 \)); in some rabbits, the submucosa was expanded up to 5 mm (figure 3B). Inflammation of the cecum varied in severity in infected rabbits from both experiments (\( P < .01 \) and \( P < .03 \); \( P = .42 \)), and heterophils were sometimes represented in high numbers (figure 3C). The mucosal cecal surface and crypt lumens were lined with surface-adherent \( E. coli \). Vascular lesions consistent with intimal damage and increased permeability included endothelial hypertrophy;
medial vacuolar degeneration; and medial, adventitial, and perivascular edema.

Coinciding with the altered clinical pathological profile, rabbit 5 in experiment 1 displayed severe lesions. Numerous adherent *E. coli* lined the ulcerated pseudomembranous cecal mucosa, as demonstrated by PNA-FISH (figure 3D and 3E). Heterophils infiltrated the submucosa and lamina propria, with abundant epithelial and vascular transcytosis. An ischemic origin for mucosal necrosis and ulceration was suggested by necrotic vessels at the base of such lesions with occlusive fibrin thrombi (figure 3F).

Renal vascular lesions in infected rabbits were characterized by fibrinoidedematous vasculopathy concentrated on interlobular arterioles and venules with intimal swelling (*P* < .01 and *P* < .002) and/or medial intravascular edema, small surface and/or subendothelial fibrin deposits, and perivascular edema (*P* < .01 and *P* < .01; *P* < .002) (figure 4). Arcuate arteries and interlobular arterioles in rabbit 5 infected with O153 (experiment 1) demonstrated marked mucinous intimal hypertrophy characterized by endothelial hypertrophy (figure 5A and 5B) that resulted in luminal constriction. In some vessels, congealed masses of mucus, fibrin, and red blood cells obstructed the lumen.

Glomerular lesions in infected rabbits included luminal constriction (*P* < .01 and *P* < .01; *P* < .003) and intracellular edema resulting in swelling of the tufts and decreased numbers of erythrocytes with increased numbers of heterophils (*P* < .01 and

**Figure 5.** A, Renal interlobular arteriole from a sham-inoculated normal rabbit. B, Renal interlobular arteriole from rabbit 5 (experiment 1) infected with O153, demonstrating mucoid intimal hypertrophy (arrow), luminal constriction with plasma proteofibrinous condensate, and expansion of perivascular connective tissue (edema). C, Normal rabbit glomerulus. D, Glomerulus of an O153-infected rabbit demonstrating global intracellular edematous swelling, decreased numbers of erythrocytes (“bloodless glomerulus”), and increased numbers of heterophils (arrows). E, Glomerulus of rabbit 5 (experiment 2) infected with O153, demonstrating mesangial deposits (arrows) and capillary thickening (arrowheads). F, Carstairs’ stain of glomerulus from an O153-infected rabbit, demonstrating small fibrin thrombi in glomerular capillaries and in periglomerular vessels (arrows). Staining was performed with hematoxylin-eosin (A–E) and Carstairs’ stain for fibrin (F). Scale bar, 40 μm (A and B), 60 μm (C, D, and E), and 80 μm (F).

**Figure 6.** Glomerulus of rabbit 5 infected with O157:H7, demonstrating global intracellular swelling and distortion of endothelial, mesangial, and podocyte cells.
Figure 7. Ultrastructural evaluation of cecum and kidney from rabbit 5 (experiment 1) infected with O153, using transmission electron microscopy. A, Cecal vessel disrupted by edema and infiltrated by leukocytes, including a granulocyte (“G”), a macrophage (“M”), and plasma cells (“P”). B, Intracellular edema in glomerular podocyte (arrows). C, Glomerular endothelial vacuolar degeneration (arrows). D, Tortuous disorganization of endothelial cytoplasm and basement membranes (arrow). E, Vacular degeneration of tubular epithelium (arrow). F, Misshapen (arrow) and fragmented (arrowhead) erythrocytes consistent with membrane weakness and thrombotic microangiopathic erythropathy, respectively. Scale bar, ∼1 μm (A, B, C, D, and E), ∼5 μm (F).

Sequence analysis of stx1AB. A scheme of the genetic loci within the 7333-bp O153 stx region is shown in figure 8. The sequence data have been submitted to GenBank (accession number AY838795). The O153 chromosomal sequence is >99% identical at the nucleotide level to that of φ 4795 from bp 1 to 4856 (encompassing genes homologous to Q, stxA, stxB, and yjhs) and remains homologous to φ 4795 through bp 6170, encompassing the lysis genes S and R (>92% identity at the nucleotide level). The region from bp 6250 to 7333, encoding the genes ant and Rz, is most homologous to the lysis gene region of the cryptic phage CP-933N from E. coli O157:H7 CDC EDL 933 (>98% identity at the nucleotide level) (GenBank accession number AE005324). The sequenced O153 region is also 96% identical to phage Stx1φ (GenBank accession number AP005153) at the nucleotide level, from bp 2918 to 6170; this phage was isolated from a human clinical O157:H7 strain, Morioka V526 [22]. The O153 region from bp 2918 to
Figure 9. Collision-induced decomposition mass spectra obtained during liquid chromatography–tandem mass spectrometry (MS/MS) analyses of commercial globrotriaosylceramide (Gb3) standard (A), extract from New Zealand White (NZW) rabbit kidney cortex (B), and extract from Dutch Belted (DB) rabbit kidney cortex (C).

Gb3 analyses. Glycolipid extracts of kidney cortex and medulla from DB and NZW rabbits were separated by TLC and migrated to the same position as the Gb3 standard (data not shown). Tandem mass spectrometers provide important structural information by inducing fragmentation of the ions to yield collision-induced decomposition (CID) spectra, in which the mass differences between signals correspond to losses of characteristic structural moieties. Gb3 is a family of compounds with a common structural feature of 3 monosaccharides but with different lipid chains and, thus, different molecular weights [25, 26]. The mass-spectrometric behavior of Gb3 molecules is characterized by 3 ions separated by 162 Da, which corresponds to the sequential loss of the 3 carbohydrate moieties [25, 26]. Thus, a compound is defined as a Gb3 by this characteristic pattern. This is illustrated in the CID spectrum from the Gb3 standard ([M+H+]⁺ at m/z = 1132.9) (figure 9A). The ions at m/z = 970.7, m/z = 808.7, and m/z = 646.5 correspond to losses of 1, 2, and 3 monosaccharides, respectively. LC-MS/MS analysis of kidney extracts from both DB and NZW rabbits revealed compounds with these mass spectral characteristics. The relevant protonated molecular ions were at m/z = 1118.9 and m/z = 1135.4 for both rabbit breeds and at m/z = 1091.3 for the DB rabbit sample, suggesting the presence of at least 3 Gb3 homologs in rabbit kidney (figure 9B and 9C).

6170 also shows 96% homology to the prophages VT1-Sakai and VT2-Sakai [23, 24], as well as to the cryptic phage CP-933V from CDC EDL 933.

The O153 Q product is most homologous to the Q proteins of H19B and φ 4795 (99% identity over 144 aa). The product of stx1A is identical to that of φ 4795 and Shigella dysenteriae, differing from StxA1 of phage H19B in 1 aa (a threonine is predicted instead of a serine at aa 67 in O153). The product of yjhS is most homologous to the YjhS protein of φ 4795 (99% identity over 645 aa). The yjhS gene is flanked by 2 open reading frames (ORFs), which are homologous to corresponding ORFs of φ 4795 (orf40, 100% identity over 75 aa; orf44, 94% identity over 59 aa). The products of S and R are most homologous to S and R of phage VT2-Sa (GenBank accession number NC_000902) [23] and the cryptic prophage CP-933V.
DISCUSSION

We report that experimental enteric infection of weaned DB rabbits with EHEC reproducibly results in significant renal lesions. All of the infected rabbits in both experiments developed renal vascular and glomerular lesions. Stx1 and/or Stx2 induce a spectrum of renal pathological changes [27–29]. The renal pathological changes in infected rabbits were similar to lesions described in humans with HUS [19, 30]. In humans, endothelial cell swelling results in thickened capillary walls and reduced occluded capillary lumens. In infected rabbits, glomerular capillary thickening, luminal constriction, and mesangial deposits were consistent features. Vascular lesions and erythrocyte fragmentation indicated microangiopathic injury. Increased numbers of leukocytes in the glomeruli have also been described in humans. Stx1 binds to circulating polymorphonuclear leukocytes [31].

EHEC-infected rabbits exhibited diarrhea and weight loss, shed bacteria, and developed enteric disease, which supports the pathogenic role of adherence and Stxs in NZW rabbits [16, 18, 32, 33]. In each experiment, 33%–40% of the rabbits developed acute disease during the first week of infection. These animals shed the highest levels of bacteria, which correlated with severity of the disease. The difference in age was not a major determinant of clinical severity. Rabbits displayed severe gastrointestinal manifestations similar to those seen in human O157:H7 infections [34, 35]. Ultrastructural evaluation of the cecum demonstrated vascular transmural edema and leukocyte infiltration. Stx translocation across intestinal epithelial cells is enhanced by neutrophil transmigration [11].

Infected rabbits developed a wide range of clinical pathological changes similar to those described in humans infected with EHEC. Incomplete forms of HUS have been reported in patients who present with anemia and/or thrombocytopenia with or without azotemia [36, 37]. Children infected with O157:H7 develop vascular injury without evidence of hematologic or biochemical renal changes [2]. The elevated BUN levels in the O153-infected rabbits and the normal creatinine levels suggested prerenal azotemia rather than renal insufficiency; however, 100% of the EHEC-infected rabbits developed renal lesions. It will be important to determine whether the clinical and pathological changes in infected rabbits are associated with decreased glomerular filtration rates and long-term sequelae.

Expression of stx genes and production of Stx is linked to bacteriophage induction. Since the amount and type of toxin produced are important for the outcome of EHEC infection, we performed sequence analysis of the Stx1-converting bacteriophage of O153. The Q gene and the stx promoter region are highly related to those of the Stx1-producing bacteriophage, H19B, suggesting that the stx genes in O153 are controlled by the 2 known regulators of stxl, the antiterminator Q and the iron regulatory protein Fur.

Gb3s are the functional binding receptors of Stx and are major components of the glycolipids in human renal tissue [21]. Gb3 is composed of 3 carbohydrates and a lipid moiety. The Stx binding specificity is contained within the terminal carbohydrate moiety of Gb3; however, the lipid portion is also required for binding [38]. In previous experiments in NZW rabbits, in which purified Stx1 was infused intravenously, it was postulated that renal lesions did not develop and that the rabbit model failed to replicate human HUS because of a lack of the Stx receptor in the kidneys [39, 40]. However, our detection of Gb3 in the kidneys of rabbits by use of LC-MS/MS, the method of choice for characterization of glycolipids, reinforces the similarities between Stx-induced disease in rabbits and its counterpart in humans. The Gb3 homologs are potential Stx targets that may play a role in the pathogenesis of renal disease in DB rabbits. Although Gb3 homologs were detected in renal tissue from both rabbit breeds, the differential tissue targeting between breeds may involve differences in the lipid moiety of the receptor [38].

The reproducibility of renal lesions in DB rabbits experimentally infected with EHEC provides an opportunity to design treatments that can reduce the renal complications of EHEC infection in humans. Future studies using DB rabbits infected with O153 or other Stx1- or Stx2-producing EHEC strains may reveal new insights into the pathophysiological events responsible for this debilitating human disease.

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