

Brief Report

HEMOLYTIC-UREMIC SYNDROME IN A SIX-YEAR-OLD GIRL AFTER A URINARY TRACT INFECTION WITH SHIGA-TOXIN-PRODUCING *ESCHERICHIA COLI* O103:H2

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IN the United States, the hemolytic-uremic syndrome of childhood typically follows gastrointestinal infection with *Escherichia coli* O157:H7.¹⁻³ It is presumed that the absorption from the gastrointestinal tract of Shiga toxins 1, 2, or both (formerly called Shiga-like toxins⁴) produced by *E. coli* O157:H7 causes microangiopathic hemolytic anemia as a result of endothelial-cell injury.⁵ Shiga-toxin-producing *E. coli* belonging to serotypes other than O157:H7 can also cause the hemolytic-uremic syndrome.^{5,6} However, even though such organisms have been implicated as causes of sporadic cases⁷ or outbreaks⁸ of gastroenteritis, they are not believed to be important causes of the hemolytic-uremic syndrome in this country.

The hemolytic-uremic syndrome occasionally follows urinary tract infections.⁹⁻¹⁴ In two cases, the syndrome was atypical: it was recurrent in one case⁹ and associated with familial hypocomplementemia in another.¹⁰ In two other reports that associated the hemolytic-uremic syndrome with urinary tract infection, *E. coli* O157:H7 was recovered from the urine of one child with hemorrhagic colitis and cystitis,¹³ and Shiga-toxin-producing *E. coli* O17:H18 was recovered from the urine and blood of a child with antecedent diarrhea.¹⁴

We describe a child who had the hemolytic-uremic syndrome but no prodromal diarrhea after a

nonbacteremic urinary tract infection with *E. coli* O103:H2 that produced Shiga toxin 1. We also describe the characteristics of the infecting organism.

CASE REPORT

A previously healthy six-year-old girl was seen after eight hours of abdominal and left-flank pain, vomiting, and dysuria. Constipation had been treated by enema on the preceding day. The child had not had diarrhea during the two weeks before evaluation.

On examination, the patient had an oral temperature of 39.8°C, a pulse rate of 120 per minute, and a blood pressure of 111/59 mm Hg. There was tenderness of the suprapubic area and left costovertebral angle. The white-cell count was 23,400 per cubic millimeter (58 percent neutrophils, 23 percent band forms, 9 percent lymphocytes, and 10 percent monocytes), the hematocrit was 40 percent, and the platelet count was 293,000 per cubic millimeter. Morphologic analysis of erythrocytes demonstrated no abnormalities. The blood urea nitrogen concentration was 18 mg per deciliter (6.4 mmol per liter), and the serum creatinine concentration was 0.5 mg per deciliter (44 μmol per liter). Urine obtained by catheterization had a specific gravity of 1.028 and a pH of 5, and dipstick analysis was positive for leukocyte esterase (++) , protein (++) , ketones (++) , and blood (+++). The urinary sediment contained 21 to 100 red cells, more than 100 white cells, 1 to 5 renal tubular cells, and 1 to 5 granular casts per high-power field. Many bacteria were observed by microscopy. Abdominal ultrasonography demonstrated attenuation of fat and a probable collection of fluid around the left kidney; the right kidney was normal. Blood was obtained for culture before treatment with ceftriaxone was begun.

The patient was admitted to the hospital with a diagnosis of pyelonephritis. On the next day, the urine culture was reported as growing pure *E. coli* at a concentration of more than 10⁵ colony-forming units per milliliter. Treatment was changed to ampicillin, in accordance with the antibiotic susceptibilities of the urinary isolate. Hydration and intravenous antibiotic therapy initially produced improvement, but on the second hospital day nonbloody diarrhea began and abdominal pain and vomiting recurred. A simultaneous laboratory evaluation demonstrated persistent leukocytosis (27,100 white cells per cubic millimeter, with 86 percent neutrophils, 3 percent band forms, 6 percent lymphocytes, and 5 percent monocytes), anemia (hematocrit of 30 percent), and thrombocytopenia (34,000 platelets per cubic millimeter). Erythrocyte fragments were seen on a blood smear. The prothrombin time, activated partial-thromboplastin time, and circulating D-dimer concentration were normal. The blood urea nitrogen concentration was 17 mg per deciliter (6.1 mmol per liter), but the serum creatinine concentration had risen to 1.0 mg per deciliter (88 μmol per liter). Stool was obtained for bacterial culture (including screening for campylobacter, *E. coli* O157:H7, salmonella, shigella, and yersinia) and an assay for *Clostridium difficile* toxin. Cultures of blood and urine were also repeated.

During the next 48 hours, progressive microangiopathic hemolytic anemia, thrombocytopenia, and uremia developed, without evidence of consumptive coagulopathy, thereby establishing a diagnosis of the hemolytic-uremic syndrome. Both blood cultures and the second urine culture remained sterile. The stool culture did not yield enteric pathogens, and fecal *C. difficile* toxin was not detected. The patient required 11 days of hemodialysis for oliguria, hypertension, and uremia, and erythrocyte and platelet transfusions for severe anemia and thrombocytopenia. Parenteral ampicillin was continued. Ultrasonography on hospital day 15 revealed a marginated lesion 15 mm in diameter in the lower pole of the left kidney, consistent with the occurrence of infarction or abscess; both kidneys had echogenic changes characteristic of the hemolytic-uremic syndrome.

The patient was sent home after 20 days of hospitalization. A

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voiding cystourethrogram was normal three weeks later, and ultrasonography six weeks after discharge demonstrated that the focal abnormality in the left kidney had resolved. As of this writing, two years later, the patient has normal blood urea nitrogen and serum creatinine concentrations, iothalamate clearance, and blood pressure, and her urine is free of protein and blood. She has not had a recurrence of either a urinary tract infection or the hemolytic-uremic syndrome.

CHARACTERISTICS OF THE INFECTING STRAIN

The urinary tract isolate was identified as *E. coli* serotype O103:H2. This isolate ferments sorbitol when plated on sorbitol MacConkey agar, is hemolytic¹⁵ and cytotoxic for Vero cells,¹⁶ adheres to HeLa

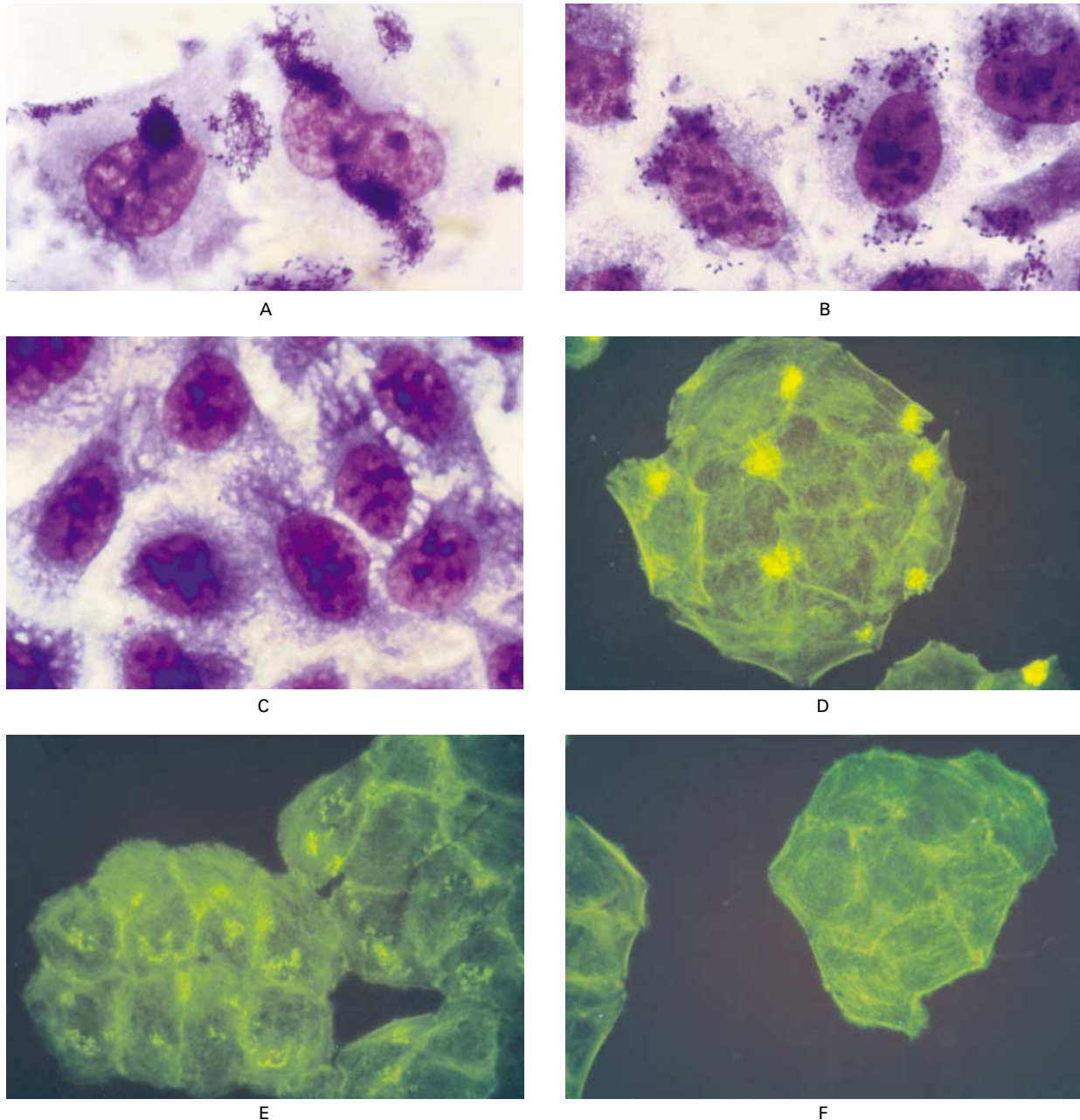


Figure 1. Adherence of Various Strains of *E. coli* to HeLa Cells.

Nontoxigenic, enteropathogenic *E. coli* O111:NM (Panel A) and *E. coli* O103:H2, which was isolated from the patient's urinary tract (Panel B), adhere to epithelial cells in a localized pattern, whereas *E. coli* HB101 (Panel C) is nonadherent, as demonstrated by Giemsa staining ($\times 1005$). Actin aggregates are present at the site of adherence of bacterial microcolonies to HeLa cells incubated with *E. coli* O111:NM (Panel D) and *E. coli* O103:H2, which was isolated from the patient's urinary tract (Panel E), as demonstrated by fluorescein-phalloidin staining ($\times 635$).¹⁷ HeLa cells incubated with *E. coli* HB101 do not have any actin aggregates (Panel F, $\times 635$).

cells in a localized pattern (Fig. 1B), and induces actin aggregation in the cells to which it adheres (Fig. 1E).^{7,17} This strain does not agglutinate galactose α 1-4galactose-coated beads or sheep or human erythrocytes,^{18,19} nor does it possess sequences homologous to the *pap* operon or the Dr-receptor-recognizing family of adhesins.^{19,20}

The infecting strain contains *stx1*, encoding Shiga toxin 1, as evidenced by Southern hybridization under stringent conditions²¹ of the *stx1* probe²² to a 9-kb *Eco*RI fragment of the bacterial DNA. The *stx2* probe²² (encoding Shiga toxin 2) failed to hybridize to sequences in the bacterial DNA. Furthermore, primers specific for *stx1* (5'GAAGAGTCCGTTGGATTACG3' and 5'AGCGATGCAGCTATTAATAA3') amplified a fragment of 130 base pairs, whereas *stx2*-specific primers (5'TTAACCACACCCACGGCAGT3' and 5'GCTCTGGATGCATCTCTGGT3') failed to amplify any *stx2* sequences.²³ Southern hybridization also showed that this strain of *E. coli* O103:H2 possesses *eaeA*, encoding intimin,²⁴ a bacterial protein that mediates the effacement of enterocytes by attached enteropathogenic *E. coli*.

DISCUSSION

A strain of *E. coli* O103:H2 producing Shiga toxin 1 was recovered from the urine of a six-year-old girl one day before the first laboratory evidence of the hemolytic-uremic syndrome appeared. Since there was no antecedent diarrhea, it is probable that urinary Shiga-toxin-producing *E. coli* O103:H2 produced the absorbed toxin that precipitated the syndrome. Diarrhea, which began at the same time as microangiopathic changes were observed on a blood smear, was more likely to have been a side effect of the broad-spectrum antibiotic used to treat the urinary tract infection than of gastrointestinal infection due to Shiga-toxin-producing *E. coli*. The demonstration of Shiga-toxin-producing *E. coli* in the urinary system in the absence of bacteremia or diarrhea before the onset of the hemolytic-uremic syndrome suggests that the human uroepithelium, like the gastrointestinal mucosa, might permit the absorption of Shiga toxin 1. Ultrasonography and voiding cystourethrography revealed no anatomical abnormalities that would predispose the patient to urinary stasis. However, the possible abscess in the lower pole of the left kidney might have provided a portal of entry for the toxin. Although it is unlikely given the sequence of events, we cannot exclude with certainty a gastrointestinal source of the absorbed toxin, because the microbiologic analysis of the stool on the second hospital day would not have detected sorbitol-fermenting *E. coli* O103:H2, since assays for fecal Shiga toxin or an analysis of recovered *E. coli* for toxigenicity was not performed.

This case demonstrates that the hemolytic-uremic syndrome can be caused by Shiga-toxin-producing

E. coli other than *E. coli* O157:H7 in the United States. The serotype and toxin genotype of the urinary *E. coli* O103:H2 isolate are identical to those of strains associated with sporadic postdiarrheal hemolytic-uremic syndrome in France.²⁵ However, the toxin genotype of this urinary isolate contrasts with that of *E. coli* O157:H7, which almost always contains genes encoding Shiga toxin 2, especially strains recovered from patients with the hemolytic-uremic syndrome.²⁶ In the United States, Shiga-toxin-producing *E. coli* O103:H2 has been isolated from cultures of feces in cattle.^{27,28} The recovery of *E. coli* O103:H2 from our patient suggests that *E. coli* O103:H2 might emerge as a pathogen in North America.

The ability of the infecting strain to adhere to epithelial cells in a localized pattern and to induce the aggregation of actin in cells to which it adheres are cardinal virulence traits of enteropathogenic *E. coli* (Fig. 1A and 1D),²⁹ and of *E. coli* O157:H7. Indeed, diarrhea was observed in French children infected with *E. coli* O103:H2²⁵ and in Italian children with the hemolytic-uremic syndrome whose serum contained antibodies against *E. coli* O103 lipopolysaccharide.³⁰ It is impossible to identify the sorbitol-fermenting strain of *E. coli* O103:H2 in a stool culture with techniques that identify *E. coli* O157:H7, which rely on screening with sorbitol MacConkey agar followed by determination of the O157 antigen. Although the infecting *E. coli* O103:H2 isolate was identified with ease in a urine specimen obtained by catheterization, this organism would probably have been interpreted by microbiology laboratories as belonging to the normal gastrointestinal flora had it been present in a stool specimen, and would not have been subjected to cytotoxicity assays (cell culture) or DNA hybridization analysis (gene probing or the polymerase chain reaction), since these studies are usually performed only in reference laboratories. New assays,^{31,32} which rapidly and specifically identify Shiga toxins produced by fecal *E. coli* by exploiting the binding of these toxins to globotriaosylceramide, should increase the ability of clinical microbiology laboratories to identify these enteric pathogens.

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