ISSN 0025-7680

MEDICINA (Buenos Aires) 53: 487-490 (1993)

HEMOLYTIC UREMIC SYNDROME: CO-INFECTION WITH TWO DIFFERENT SEROTYPES OF SHIGA-LIKE TOXIN PRODUCING *ESCHERICHIA COLI*

MARTA RIVAS[1], L. VOYER[2], MONICA TOUS[1], NELIDA LEARDINI[1], MARIA F. DE MENA[1], RAQUEL WAINSZTEIN[2], RAQUEL CALLEJO[1], VALERIA PRADO[3], NORMA BINSZTEIN[1]

[1] Instituto Nacional de Microbiología Carlos G. Malbrán; [2] Hospital General de Niños Pedro de Elizalde, Buenos Aires; [3] División Ciencias Médicas Oriente, Facultad de Medicina, Universidad de Chile, Santiago de Chile.

Summary We report a case of a child who developed Hemolytic Uremic Syndrome in whom two Shiga-like toxin (SLT) - producing *Escherichia coli* strains of different serotypes and genotypes, were simultaneously isolated from stools. In addition, one of these strains represented a new toxin producing serotype. Strain 1 belonged to serotype O157: H7, biotype D, produced SLT II and was susceptible to all antibiotics tested. This strain hybridized with gene probes for SLT II, fimbrial adhesion (EHEC factor) and attaching and effacing factor (eae). Strain 2 belonged to serotype 025: K2: H2, produced SLT II and had a multiresistant antibiotic susceptibility pattern. This strain hybridized with the EHEC gene probe but not with SLT I, SLT II and eae gene probes. Free fecal SLT II cytotoxin was detected in stools of the child and his father, suggesting that the infection may have been acquired from a household contact.

Hemolytic Uremic Syndrome (HUS) is the most common cause of acute renal failure in Argentina. Around 250 new cases are reported every year, with an estimated annual incidence rate of 7.8/100000 children under 5 years of age, representing the highest incidence rate in the world[10, 14].

For many years the etiology of HUS was obscure and HUS was considered to be idiopathic. In 1985 Karmali and colleagues established an association between HUS and verotoxin (Shiga-like toxin) producing *Escherichia coli* infection in children from Canada[4]. Since then, this association has been reported in many countries including Argentina[3, 10].

We report HUS in a child, in whom two Shiga-like toxin-producing *E. coli* strains of different serotypes and genotypes were simultaneously isolated from stools. One of these strains represents a new toxin producing serotype.

Received: 28-VII-1993 Accepted: 29-IX-1993

Postal address: Servicio Fisiopatogenia, Instituto Nacional de Microbiología, Avenida Vélez Sarsfield 563, 1281 Buenos Aires, Argentina.

Material and methods

Case report

A 14 month-old male was well until 8 days prior to hospital admission when he developed vomiting, bloody diarrhea and abdominal pain. He was afebrile. The child was referred to the Hospital General de Niños Pedro de Elizalde with watery diarrhea for treatment of moderate dehydration. He had not received antibiotics and a review of possible contacts revealed that his father had diarrhea the previous week.

Laboratory studies on admission revealed: serum sodium concentration 118 and potassium 2.1 mEq/l; peripheral leuckocyte count 26900/mm^3; platelet count 136000/mm^3 and hematocrit 41%.

Dehydration was treated with a standard WHO oral rehydration solution.

No antibiotic was used. During the next 3 days the watery diarrhea persisted. On day 3 there was an abrupt fall in hematocrit to 20% and schistocytes and anisopoikilocytosis were seen on a peripheral smear. Urine output and blood pressure remained normal. The urinalysis showed glucose 1+, protein 1+, hemoglobin 3+, and numerous red blood cell casts were seen by microscopy. The serum creatinine concentration was 0.71 mg/dl and the glomerular filtration rate was 61.9 ml/minute/1.73 m^2, slightly low for his age.

HUS was diagnosed and 10 ml/kg of packed red blood cells were given.

The hospital course was benign, and he was discharged on day 20.

At follow-up eight months later he was asymptomatic and blood pressure, urine output, serum creatinine and urine sediment were normal.

Sample assays

Stool samples from the patient and his father were obtained 8 and 17 days, 3 and 6 months after onset of diarrhea. Samples were processed for detection of free fecal Shiga-like toxins, for identification and characterization of Shiga-like toxin-producing *E. coli*, and for detection of common enteropathogens[1, 6].

Identification and biotyping of Shiga-like toxin-producing *E. coli* in stools was performed as previously described[6, 7].

Antibiotic susceptibility patterns were assayed by the Kirby Bauer method[1] for ampicillin, carbenicillin, cephalotin, chloramphenicol, streptomycin, gentamycin, nalidixic acid, colistin, and tetracyclin. *E. coli* strains were tested for presence of different known O and H antigens by standard methods at the International Center of Escherichia and Klebsiella, Statens Seruminstitut, Copenhague, Denmark[15].

E. coli virulence factors including cytotoxins, fimbrial adhesion (EHEC factor), and attaching and effacing factor (eae), were determined for all Shiga-like toxin producing strains by biotin-d-UTP labeled gene probe under stringent conditions[4]. Gene probes used were Shiga-like toxin I probe, a BamH1 fragment of 1.1-kbp cloned from the pJN37-19 plasmid; Shiga-like toxin II probe, a SmaI-PstI fragment of 0.84-kbp cloned from the pNN110-18 plasmid; EHEC probe, a HindIII fragment of 3.4-kbp cloned from the pCVD419 plasmid; eae probe, a SalI-KpnI fragment of 1-kbp cloned from pCVD434[5, 13, 18].

Cytotoxic activity of fecal extracts, bacterial supernatants and periplasmic cell extracts was assayed on Vero 76 cells (ATCC CCL81) as previously described[5]. Specific toxin activity was assigned by neutralization tests using Shiga-like toxin I and II specific monoclonal antibodies (MAb 13C4 and BC5BB12 respectively), provided by NA Strockbine, Center for Infectious Disease, Atlanta, Georgia, USA.

Results

The stool sample obtained from the patient on day 8 was positive for cytotoxin-producing *E. coli*. We did not isolate a similar organism from any of the four stool samples obtained from the father.

Two *E. coli* strains, O157: H7 and O25: K2: H2 were isolated from the same stool sample of the patient. Both strains produced Shiga-like toxin II only. The O157: H7 strain corresponded to biotype D, was susceptible to all the antibiotics tested, and was gene probe positive for SLT II, EHEC and eae. The O25: K2: H2 strain, a previously non-described Shiga-like toxin-producing serotype, was a sorbitol fermentor, was glucuronidase and lysine decarboxylase positive, and had a multi-resistant antibiotic susceptibility pattern. This strain was gene probe positive for EHEC but not for eae, SLT I and SLT II. Production of Shiga-like toxin II from this strain was confirmed by cytotoxic and neutralization assay.

The O157: H7 strain produced less cell-bound and free toxin than the O25: K2: H2. Polymyxin-releasable cell extract and supernatant of 5-h Penassay broth cultures[6] of the *E. coli* strain O25: K2: H2 produced twenty fold higher SLT II titers than the O157: H7 strain.

Free fecal toxin II activity was detected from both the patient and his father's stools 8 days after the onset of diarrhea. Toxin activity was not detected in any of the 3 stools obtained at follow-up.

Discussion

This is the first report of HUS with co-infection by two different serotypes of a Shiga-like toxin producing *E. coli*. We have also shown that one of the SLT producing strains belongs to a new serotype, not previously associated with HUS.

The sequence of events that begins with the production of SLT and ends with the development of HUS is still unknown.

The cytotoxin released into the circulation is taken up by the vascular endothelial cells, with the globotriosyl ceramide (Gb3) behaving as the func-

tional receptor, this would be the central event in the HUS pathogenesis. SLT is responsible for the damage in endothelial cells, it decreases the synthesis and release of prostacyclins and it enhances the release of von Willebrand factor multimers which cause platelet aggregation[2].

Injury to renal endothelial cells, intravascular coagglutination, and the deposition of fibrin and platelets all within the glomerulus, would produce a decrease in blood flow and subsequent renal failure.

Regarding our findings, one speculation is that the two antigenically different cytotoxins produced by both infecting organisms could differ in receptor affinities and thereby attach to different sites. This could potentially cause more endothelial damage; however, our patient was only mildly affected.

On the other hand, both serotypes of SLT producing *E. coli* have the fimbrial adhesion factor and one of them (*E. coli* O157: H7 strain) has the eae factor responsible for the attaching and effacing of the intestinal epithelial cell microvilli. This would allow the passage of bacterial products which are normally excluded from the circulation. One of them is the Lipopolysaccharide (LPS), endotoxin which may produce a Schwarztman reaction with the development of thrombocytopenia, hemolytic anemia and renal cortical necrosis, which characterizes the HUS.

It has been demonstrated in vitro that SLT, potent inhibitor of protein synthesis, may improve the procoaggulant effects of LPS or LPS-elicited interleukin - 1β (IL-1β) and tumor necrosis factor alpha (TNF-α) over the endothelial cells. Furthermore, it would react in a synergic way with TNF-α enhancing its cytotoxic activity[11].

The prevalence of the serotypes O157: H7 and O157: NM associated with hemorrhagic colitis and HUS has been reported in different regions of the world[12, 17]. The high frequency of this association may depend on the easiness to identify these serotypes in clinical microbiology laboratories due to their biochemical characteristics of sorbitol nonfermenters compared with the other *E. coli* serotypes.

The detection of the new serotype O25: K2: H2 confirms the need for using a combination of techniques when searching for cytotoxin-producing organisms. It is important to note that this new cytotoxin-producing *E. coli* would have been missed if only gene probe or classical serotyping techniques had been used for detection.

The two strains isolated differed in their antigenic characteristics, their antibiotic susceptibility pattern and the magnitude of toxin production. The O25: K2: H2 strain did not hydridize with SLT II gene probe despite showing toxin activity neutralizable by MAb BC5BB12. This result indicates that the gene sequence of the SLT II probe used, consisting in the Small-PstI fragment of 0.84-kpb cloned from the pNN110-18 plasmid, does not have homology with the gene that codes for toxin activity in this strain.

Finally, the detection of free fecal SLT II in the father, who had had diarrhea the week prior to the onset of illness of his son, suggests that the child probably acquired the infection within the household environment. The finding of SLT produced *E. coli* in family members of affected children is common in Argentina[9] and may play a role in the high incidence rate of HUS. Food-borne transmition such as meat[16] and unpasteurized milk must also be taken into consideration.

Acknowledgments: We are grateful to Dr. Ida and Fritz ϕrskov of the Statens Seruminstitut, Copenhague, Denmark for the serotyping of the *Escherichia coli* strains. The technical assistance of Fabian Pardon, Monica Prieto and Ana Garbini is acknowledged.

Resumen

Sindrome urémico hemolítico: coinfección con cepas de Escherichia coli *de distintos serotipos productoras de toxina símil-Shiga*

Se presenta el caso de un niño de 14 meses que desarrolló Sindrome Urémico Hemolítico después de un período prodrómico con vómitos y diarrea mucosanguinolenta del cual se aislaron 2 cepas de *E. coli* productoras de toxina simil-Shiga (SLT) de diferente serotipo y genotipo. Una de las cepas correspondió al serotipo O157: H7, biotipo D, productora de SLT II y susceptible a todos los antibióticos probados. Esta cepa hibridizó con las sondas genéticas para SLT II; la fimbria de adherencia (factor EHEC) y para el factor de fijación y disolución del borde en cepillo ("*E. coli* attaching and effacing" eae).

La otra cepa correspondió al serotipo O25: K2: H2 productora de SLT II en los ensayos de neutralización de efecto citotóxico sobre células

VERO utilizando anticuerpos monoclonales, pero negativa en los ensayos de hibridización con las sondas para SLT I y SLT II y el factor eae. Sólo fue positiva por hibridización con la sonda para la fimbria de adherencia. Esta cepa presentó además un patrón de multiresistencia a los antibióticos probados. Por otra parte, la cepa de *E. coli* del serotipo O25: K2: H2 produjo niveles tanto de toxina libre como asociada a células, 20 veces superiores a la cepa del serotipo O157: H7.

Esta es la primera publicación de un caso de SUH en el cual se detectó una cepa de *E. coli* del serotipo O25: H2 productora de SLT. Además el hecho de no hibridizar con la sonda genética para SLT II, a pesar de tener una actividad citotóxica neutralizable por el anticuerpo monoclonal (MAb BC5BB12) indicaría que la secuencia que codifica para SLT II no presenta homología con la sonda utilizada.

La detección de SLT libre tanto en la materia fecal del niño como en la de su padre (quien padeció diarrea una semana antes), indicaría que el niño adquirió la infección en el entorno familiar.

References

1. Balows A, Hausler WJ, Hermann KL, Isenberg HD, Shadomy HJ, (eds.): Manual of Clinical Microbiology, 5th ed, American Society for Microbiology, Washington DC, 1991.
2. Cleary TG, Lopez EL: The Shiga-like toxin-producing *Escherichia coli* and hemolytic uremic syndrome. *Pediatr Infect Dis J* 8: 720, 1989.
3. Cordovez A, Prado V, Maggi L, et al: Enterohemorrhagic *Escherichia coli* associated with Hemolytic Uremic Syndrome in Chilean children. *J Clin Microbiol* 30: 2153, 1992.
4. Gicquelais KG, Baldini MM, Martinez J, et al: Practical and economical methods for using biotinylated DNA probes with bacterial colony blots to identify diarrhea-causing *Escherichia coli*. *J Clin Microbiol* 28: 2485, 1990.
5. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H: The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis* 151: 772, 1985.
6. Karmali MA, Petric M, Lim C, Cheung R, Arbus GS: Sensitive method for detecting low numbers of Verotoxin-producing *Escherichia coli* in mixed cultures by use of colony sweeps and polymyxin extraction of Verotoxin. *J Clin Microbiol* 22: 614, 1985.
7. Krishnan C, Fitzgerald VA, Dakin SJ, Behme RJ: Laboratory investigation of outbreak of hemorrhagic colitis caused by *Escherichia coli* 0157: H7. *J Clin Microbiol* 25: 1043, 1987.
8. Levine MM, Xu J, Kaper JB, et al: A DNA probe to identify enterohemorrhagic *Escherichia coli* 0157: H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. *J Infect Dis* 156: 175, 1987.
9. Lopez EL, Diaz M, Devoto S, et al: Evidence of infection with organisms producing Shiga-like toxins in household contacts of children with the Hemolytic Uremic Syndrome. *Pediat Infect Dis J* 10: 20, 1991.
10. Lopez EL, Diaz M, Grinstein S, et al: Hemolytic Uremic Syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. *J Infect Dis* 160: 469, 1989.
11. Louise CB, Obrig TG: Shiga Toxin-Associated Hemolytic Uremic Syndrome: Combined Cytotoxic Effects of Shiga Toxin and Lipopolysaccharide (Endotoxin) on Human Vascular Endothelial Cells in Vitro. *Infect Immun* 60: 1536, 1992.
12. Neill Ma, Tarr PI, Clausen CR, Christie DL, Hickman RO: *E. coli* O157: H7 as the predominant pathogen associated with hemolytic uremic syndrome: a prospective study in the Pacific Northwest. *Pediatrics* 80: 37, 1987.
13. Newland J, Neill R: DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. *J Clin Microbiol* 26: 1292, 1988.
14. Novillo AA, Voyer LE, Cravioto R, et al: Haemolytic uraemic syndrome associated with faecal cytotoxin and verotoxin neutralizing antibodies. *Pediatr Nephrol* 2: 288, 1988.
15. Φrskov F, Φrskov I: Serotyping of *Escherichia coli*. *In:* Methods in Microbiology, Vol. 14, T Bergan, (ed.), Academic Press, London, 1984, p 43.
16. Φrskov F, Φrskov I, Villar JA: Cattle as reservoir of verotoxin-producing *Escherichia coli* O157: H7. *Lancet* 1: 276, 1987.
17. Rivas M, Voyer LE, Tous M, Leardini NA, de Mena MF, Quadri B, Wainsztein R: Detección de *Escherichia coli* O157 productor de verotoxina en pacientes con síndrome urémico hemolítico. *Medicina (Bs Aires)* 50: 571, 1990.
18. Sherman P, Soni R, Karmali MA: Attaching and effacing adherence of Vero cytotoxin-producing *Escherichia coli* to rabbit intestinal epithelium in vivo. *Infect Immun* 56: 756, 1988.