

# Basic Fibroblast Growth Factor and Shiga Toxin–O157:H7–Associated Hemolytic Uremic Syndrome

PHILLIP I. TARR

*Division of Gastroenterology, Children's Hospital and Regional Medical Center and the Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington.*

The hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy that occurs in about 15% of children infected with *Escherichia coli* O157:H7 and in an unknown fraction of cases after infection with other Shiga toxin (Stx)–producing *Escherichia coli* (STEC). HUS is characterized by nonimmune hemolytic anemia, thrombocytopenia, and renal insufficiency; its onset is approximately 1 wk after the first episode of diarrhea. STEC infections are rarely bacteremic; it is, therefore, presumed that HUS results from vascular and renal injury after absorption of Stx from the gut. Stx have diverse effects on eukaryotic cells (1), and the evidence for their role in mediating diarrhea-associated HUS is strong. There are three major pathophysiologic phases of HUS. Even though the exact borders between the phases cannot be distinguished with precision, this compartmentalization is useful in assessing the response of the host to infection with STEC and in appreciating the significance of the paper by Ray *et al.* (2) in this issue of *JASN*.

Phase I (the pre-HUS phase) occurs during the first several days of illness, during which time intestinal and extraintestinal, and particularly vascular, injury has started but renal injury has yet to evolve. Patients are rarely investigated prospectively at this stage. Even though considerable *in vitro* and some animal data suggest a synergistic role for inflammatory mediators and Stx in the generation of host cell and organ damage (reviewed in 3), there have not been compelling data that an early host inflammatory response is a determinant of outcome (HUS *versus* resolution of infection without renal injury). However, emerging data suggest that before the onset of azotemia, and even in children in whom renal injury does not develop, there is vascular “activation.” Specifically, there are elevations of circulating soluble Fas-ligand and soluble Fas (3), interleukin-1 receptor antagonist (4), transforming growth factor  $\beta$ -1 (5), platelet activating factor (Smith JM, *et al.*, unpublished data), degraded von Willebrand factor multimers (6), and plasma factors that demonstrate thrombin generation, intravascular fibrin accretion, and fibrinolysis inhibition (7). Some of these abnormalities can be documented in individual children

before there is azotemia or considerable elevation in urinary  $\beta_2$ -microglobulin and *N*-acetyl- $\beta$ -glucosaminidase concentrations (6,7).

The vascular target of the toxins produced by STEC in the infected host during phase I, when it is likely that the bulk of host injury is initiated, remains unknown. On the basis of *in vitro* data, the cells that are injured by Stx could be endothelial (8–21), monocytic (22–27), or both in origin. There is histopathologic evidence of endothelial cell injury in childhood HUS (6,28–31) and in primates after Stx infusion (32), but the less abundant monocyte might also be important in the pathogenesis of thrombotic disorders in response to host injury of infectious origin (33). Additionally, recent intriguing human data suggest that polymorphonuclear leukocytes (34,35) and platelets (36) that have been activated by Stx might also cause host injury. Also, direct renal injury by Stx is plausible, on the basis of *in vitro* (37–49), animal (40), and human (41) data. Whatever the cellular target of Stx, the treatment of this first phase of HUS is, at present, largely supportive. Physicians should avoid antibiotics (42) and antimotility agents (43), admit infected patients to hospital, and assure appropriate hydration via parenteral fluids (44).

Patients enter the second phase of HUS, the nadir phase, after hemolytic anemia, thrombocytopenia, and renal insufficiency have ensued. Most patients in phase II no longer have detectable STEC in their stool by this point of illness (45), and it is presumed that the multiple physiologic derangements observed during acute HUS are the consequences of the antecedent toxemia. Phase II patients are usually managed by the nephrologist, the intensivist, or both, and the mainstays of therapy are dialysis if indicated, BP control, transfusions, and vigilant anticipation of, and appropriate treatment for, surgically remediable complications (46).

The return of the platelet count toward normal (*i.e.*, a spontaneously rising platelet count in the absence of transfusions) and the indicators of the return of renal function (a spontaneously falling serum creatinine concentration or the return of urinary output after a period of anuria) mark the third phase of HUS. The sequential attainment of hematologic and then nephrologic resolution in most cases suggests that vascular improvement begins before renal improvement is manifest. Indeed, resolution of fibrinolysis inhibition has been postulated to herald, and possibly to lead to, the resolution of HUS (47).

Correspondence to: Phillip I. Tarr, M.D., Division of Gastroenterology CH-24, Children's Hospital and Regional Medical Center, 4800 Sand Point Way N.E., Seattle, WA 98105. Phone: 206-526-2521; Fax: 206-526-2221; E-mail: tarr@u.washington.edu

1046-6673/1303-0817

Journal of the American Society of Nephrology

Copyright © 2002 by the American Society of Nephrology

Ray *et al.* (2) make a new and important contribution to our understanding of the mechanism of resolution of HUS. They identified children with HUS early in phase II of illness and followed them systematically and prospectively through phase III. They focused on basic fibroblast growth factor (bFGF) and compared the time course of circulating and urinary levels of bFGF with a panel of inflammatory mediators. Though this study was intentionally limited to the children who were not oligoanuric, this report has multiple practical and theoretical implications that warrant individual comment.

#### *Urinary bFGF Is of Renal Origin during HUS*

In the setting of elevated and presumably rising creatinines, the urinary bFGF levels rose. This suggests a renal and not a circulating origin for this protein, especially as the concentration was normalized to the urine creatinine. bFGF is found in human renal vascular endothelial cells, as expected, as well as in glomerular parietal epithelial cells, tubular cells, and podocytes (48). Experimental data suggest that bFGF may be an autocrine factor that promotes recovery of renal tubular epithelial cells to injury (49), and bFGF is the primary mediator of autocrine-induced growth regulation in response to inflammatory factors (50). bFGF binds to glomerular and tubular-interstitial heparan sulfate proteoglycans in HUS associated with HIV infection and has been postulated to play a role in glomerular and tubular regeneration in this disorder (51). On the other hand, animal data indicate that bFGF might mediate renal injury, as described below. Clearly, the elucidation of the role of bFGF in renal injury and repair appears to be a worthwhile avenue of investigation.

#### *Prolonged Elevations of Circulating bFGF Demonstrate Prolonged Endothelial Injury in STEC-Related HUS*

Currently available data suggest that circulating bFGF is solely or predominantly of endothelial origin. It is not secreted in the basal state, but it is released from the endothelium in response to injury. It is interesting that the level of circulating bFGF remains elevated as recovery ensues and suggests some degree of continuing endothelial perturbation well after the initial toxic insult has passed and recovery has commenced. This putative late stimulation or injury of the endothelium is unexpected in view of the usually complete return to normal function of the kidney after nonoligoanuric HUS, and it raises the possibility that there is perpetuation of vascular injury well after the toxemia from a gastrointestinal STEC infection has ended. Alternatively, there could be a late defect in clearance of this molecule by recovering kidneys after HUS.

#### *Circulating Concentrations of bFGF Rise as Recovery from HUS Occurs*

Ideally, the data should have been related to the point of resolution in each individual child via a time-to-event model such as the proportional hazards Cox regression model (52). In individual cases, however, it is not possible to predict when resolution will occur. Therefore, the aggregate analysis reported by Ray *et al.* (2) is an acceptable alternative approach

for this first ever analysis. The pathophysiologic implication of their findings is that bFGF is a candidate mediator of the resolution phase and might even be useful as a therapeutic. This role for bFGF is plausible because, like vascular endothelial growth factor (VEGF), bFGF promotes a variety of host reparative responses such as angiogenesis, neovascularization, and renal growth (53), which could attenuate or reverse the thrombotic occlusive component of HUS. In this light, it is interesting to note that VEGF protects against renal infarction (54) and accelerates renal recovery (55) in antibody-mediated thrombotic microangiopathy in rats.

However, bFGF might also have a potentially sinister side. In rats, bFGF increases matrix formation (56,57). bFGF also mediates cytotoxicity (58) and mesangial cell mitogenesis (59) in anti-Thy 1.1 membranoproliferative glomerulonephritis, mesangial cell proliferation after subnephritogenic infusion of anti-Thy 1.1 (56), and podocyte damage in passive Heymann nephritis (60).

#### *Intraintestinal Shiga Toxin Neutralization during HUS is Futile*

Ray *et al.* (2) report that Synsorb-Pk fails to attenuate the course of postdiarrheal HUS, though the detailed analysis of this intervention study is beyond the scope of their manuscript. However, it is noteworthy that this apparently safe oral toxin binder conferred no advantage to the treated group. HUS is almost certainly a posttoxemic event, and vascular injury is already underway within 4 d of the onset of diarrhea, before the case definition of HUS is fulfilled (6,7).

Reversal of the pathophysiologic cascade leading to host, and in particular, renal injury, and not antibacterial (42) or antitoxin therapy, offers the best hope for attenuating the effects of established infection. Ray *et al.* (2) are to be commended on their ability to obtain solid pathophysiologic data from an unfortunately disappointing, but nonetheless well-designed and performed, intervention trial. Studies such as this could point the way for future attempts to ameliorate the course of post-*Escherichia coli* O157:H7 HUS by repairing the vascular-occlusive component of Stx-mediated HUS.

#### References

1. Nakao H, Takeda T: *Escherichia coli* Shiga toxin. *J Nat Toxins* 9: 299–313, 2000
2. Ray P: Basic fibroblast growth factor among children with diarrhea-associated hemolytic uremic syndrome. *J Am Soc Nephrol* 13: 699–707, 2002
3. Proulx F, Seidman EG, Karpman D: Pathogenesis of Shiga toxin-associated hemolytic uremic syndrome. *Pediatr Res* 50: 163–171, 2001
4. Proulx F, Turgeon JP, Litalien C, Mariscalco MM, Robitaille P, Seidman E: Inflammatory mediators in *Escherichia coli* O157:H7 hemorrhagic colitis and hemolytic-uremic syndrome. *Pediatr Infect Dis J* 17: 899–904, 1998
5. Proulx F, Litalien C, Trugeon JP, Mariscalco MM, Seidman E: Circulating levels of transforming growth factor-beta1 and lymphokines among children with hemolytic uremic syndrome. *Am J Kidney Dis* 35: 29–34, 2000

6. Tsai HM, Chandler WL, Sarode R, Hoffman R, Jelacic S, Habeeb RL, Watkins SL, Wong CS, Williams GD, Tarr PI: von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Res* 49:653–659, 2001
7. Chandler WL, Jelacic S, Boster DR, Ciol MA, Williams GD, Watkins SL, Igarashi T, Tarr PI: Prothrombotic Coagulation Abnormalities Preceding the Hemolytic Uremic Syndrome. *N Engl J Med* 346: 23–32, 2002
8. Jacewicz MS, Acheson DW, Binion DG, West GA, Lincicome LL, Fiocchi C, Keusch GT: Responses of human intestinal microvascular endothelial cells to Shiga toxins 1 and 2 and pathogenesis of hemorrhagic colitis. *Infect Immun* 67:1439–1444, 1999
9. Ohmi K, Kiyokawa N, Takeda T, Fujimoto J: Human microvascular endothelial cells are strongly sensitive to Shiga toxins. *Biochem Biophys Res Commun* 251: 137–141, 1998
10. Louise CB, Tran MC, Obrig TG: Sensitization of human umbilical vein endothelial cells to Shiga toxin: Involvement of protein kinase C and NF-kappaB. *Infect Immun* 65: 3337–3344, 1997
11. Kaye SA, Obrig TG: Effect of TNF-alpha, shiga toxin and calcium ionophore on Weibel-Palade body content of endothelial cells: Possible implications for the hemolytic uremic syndrome. *Thromb Res* 79: 415–421, 1995
12. Louise CB, Kaye SA, Boyd B, Lingwood CA, Obrig TG: Shiga toxin-associated hemolytic uremic syndrome: Effect of sodium butyrate on sensitivity of human umbilical vein endothelial cells to Shiga toxin. *Infect Immun* 63: 2766–2769, 1995
13. Louise C, Obrig T: Human renal microvascular endothelial cells as a potential target in the development of the hemolytic uremic syndrome as related to fibrinolysis factor expression, in vitro. *Microvasc Res* 47: 377–387, 1994
14. Kaye SA, Louise CB, Boyd B, Lingwood CA, Obrig TG: Shiga toxin-associated hemolytic uremic syndrome: Interleukin-1 beta enhancement of shiga toxin cytotoxicity toward human vascular endothelial cells in vitro. *Infect Immun* 61: 3886–3891, 1993
15. Obrig TG, Louise CB, Lingwood CA, Boyd B, Barley-Maloney L, Daniel TO: Endothelial heterogeneity in shiga toxin receptors and responses. *J Biol Chem* 268: 15848–15848, 1993
16. 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: 1536–1543, 1992
17. Louise CB, Obrig TG: Shiga toxin-associated hemolytic-uremic syndrome: Combined cytotoxic effects of Shiga toxin, interleukin-1 beta, and tumor necrosis factor alpha on human vascular endothelial cells in vitro. *Infect Immun* 59: 4173–4179, 1991
18. Tesh VL, Burris JA, Owens JW, Gordon VM, Wadolowski EA, O'Brien AD, Samuel JE: Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. *Infect Immun* 61: 3392–3402, 1993
19. Obrig TG, Del Vecchio PJ, Brown JE, Moran TP, Rowland BM, Judge TK, Rothman SW: Direct cytotoxic action of Shiga toxin on human vascular endothelial cells. *Infect Immun* 56: 2373–2378, 1988
20. Kita E, Yunou Y, Kurioka T, Harada H, Yoshikawa S, Mikasa K, Higashi N: Pathogenic mechanism of mouse brain damage caused by oral infection with Shiga toxin-producing *Escherichia coli* O157:H7. *Infect Immun* 68: 1207–1214, 2000
21. Pijpers AH, van Setten PA, van den Heuvel LP, Assmann KJ, Dijkman HB, Pennings AH, Monnens LA, van Hinsbergh VW: Verocytotoxin-induced apoptosis of human microvascular endothelial cells. *J Am Soc Nephrol* 12: 767–778, 2001
22. Foster GH, Armstrong CS, Sakiri R, Tesh VL: Shiga toxin-induced tumor necrosis factor alpha expression: Requirement for toxin enzymatic activity and monocyte protein kinase C and protein tyrosine kinases. *Infect Immun* 68: 5183–5189, 2000
23. Zhang HM, Ou ZL, Yamamoto T: Anisodamine inhibits shiga toxin type 2-mediated tumor necrosis factor-alpha production in vitro and in vivo. *Exp Biol Med (Maywood)* 226: 597–604, 2001
24. Zhang HM, Ohmura M, Gondaira F, Yamamoto T: Inhibition of Shiga toxin-induced tumor necrosis factor-alpha production and gene expression in human monocytic cells by CV6209. *Life Sci* 68: 1931–1937, 2001
25. Sakiri R, Ramegowda B, Tesh VL: Shiga toxin type 1 activates tumor necrosis factor-alpha gene transcription and nuclear translocation of the transcriptional activators nuclear factor-kappaB and activator protein-1. *Blood* 92: 588–566, 1998
26. Ramegowda B, Tesh VL: Differentiation-associated toxin receptor modulation, cytokine production, and sensitivity to Shiga-like toxins in human monocytes and monocytic cell lines. *Infect Immun* 64: 1173–1180, 1996
27. Barrett TJ, Potter ME, Strockbine NA: Evidence for participation of the macrophage in Shiga-like toxin II-induced lethality in mice. *Microb Pathog* 9: 95–103, 1990
28. Inward CD, Howie AJ, Fitzpatrick MM, Rafaat F, Milford DV, Taylor CM: Renal histopathology in fatal cases of diarrhoea-associated haemolytic uraemic syndrome. *British Association for Paediatric Nephrology. Pediatr Nephrol* 11: 556–559, 1997
29. Richardson SE, Karmali MA, Becker LE, Smith CR: The histopathology of the hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. *Hum Pathol* 19: 1102–1108, 1988
30. Riella MC, Ray CG, Hickman RO, Striker GE, Slichter SJ, Harker L, Quadracci LJ: Renal microangiopathy of the hemolytic-uremic syndrome in childhood. *Nephron* 17: 188–203, 1976
31. Habib R: Pathology of the hemolytic uremic syndrome, In: *Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura*, edited by Kaplan BS, Trompeter RS, Moake JL, New York, Marcel Dekker, Inc, 1992, pp 315–353
32. Taylor FB Jr, Tesh VL, DeBault L, Li A, Chang AC, Kosanke SD, Pysher TJ, Siegler RL: Characterization of the baboon responses to Shiga-like toxin: Descriptive study of a new primate model of toxic responses to Stx-1. *Am J Pathol* 154: 1285–1299, 1999
33. Vallet B, Wiel E: Endothelial cell dysfunction and coagulation. *Crit Care Med* 29: S36–S41, 2001
34. Te Loo DM, van Hinsbergh VW, van den Heuvel LP, Monnens LA: Detection of verocytotoxin bound to circulating polymorphonuclear leukocytes of patients with hemolytic uremic syndrome. *J Am Soc Nephrol* 12: 800–806, 2001
35. Te Loo DM, Monnens LA, van Der Velden TJ, Vermeer MA, Preyers F, Demacker PN, van den Heuvel LP, van Hinsbergh VW: Binding and transfer of verocytotoxin by polymorphonuclear leukocytes in hemolytic uremic syndrome. *Blood* 95: 3396–3402, 2000
36. Karpman D, Papadopoulou D, Nilsson K, Sjogren AC, Mikaelsson C, Lethagen S: Platelet activation by Shiga toxin and circu-



- latory factors as a pathogenetic mechanism in the hemolytic uremic syndrome. *Blood* 97: 3100–3108, 2001
37. Nakamura A, Johns EJ, Imaizumi A, Yanagawa Y, Kohsaka T: Activation of beta(2)-adrenoceptor prevents Shiga toxin 2-induced TNF-alpha gene transcription. *J Am Soc Nephrol* 12: 2288–2299, 2001
  38. Katagiri YU, Mori T, Nakajima H, Katagiri C, Taguchi T, Takeda T, Kiyokawa N, Fujimoto J: Activation of Src family kinase yes induced by Shiga toxin binding to globotriaosyl ceramide (Gb3/CD77) in low density, detergent-insoluble microdomains. *J Biol Chem* 274: 35278–35282, 1999
  39. Hughes AK, Stricklett PK, Kohan DE: Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells. *Kidney Int* 54: 426–437, 1998
  40. Shibolet O, Shina A, Rosen S, Cleary TG, Brezis M, Ashkenazi S: Shiga toxin induces medullary tubular injury in isolated perfused rat kidneys. *FEMS Immunol Med Microbiol* 18: 55–60, 1997
  41. Uchida H, Kiyokawa N, Horie H, Fujimoto J, Takeda T: The detection of Shiga toxins in the kidney of a patient with hemolytic uremic syndrome. *Pediatr Res* 45: 133–137, 1999
  42. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI: The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 342: 1930–1936, 2000
  43. Bell B, Griffin P, Lozano P, Christie D, Kobayashi J, Tarr P: Predictors of hemolytic uremic syndrome in children during a large outbreak of *Escherichia coli* O157:H7 infections. *Pediatrics* 100: E12, 1997
  44. Tarr PI, Neill MA: *Escherichia coli* O157:H7. *Gastrol Clin NA* 30: 735–751, 2001
  45. Tarr PI, Neill MA, Clausen CR, Watkins SL, Christie DL, Hickman RO: *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: Importance of early cultures in establishing etiology. *J Infect Dis* 162: 553–556, 1990
  46. Tapper D, Tarr P, Avner E, Brandt J, Waldhausen J: Lessons learned in the management of hemolytic uremic syndrome in children. *J Pediatr Surg* 30: 158–163, 1995
  47. Bergstein JM, Riley M, Bang NU: Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. *N Engl J Med* 327: 755–759, 1992
  48. Floege J, Hudkins KL, Eitner F, Cui Y, Morrison RS, Schelling MA, Alpers CE: Localization of fibroblast growth factor-2 (basic FGF) and FGF receptor-1 in adult human kidney. *Kidney Int* 56: 883–897, 1999
  49. Anderson RJ, Ray CJ: Potential autocrine and paracrine mechanisms of recovery from mechanical injury of renal tubular epithelial cells. *Am J Physiol* 274: F463–F472, 1998
  50. Francki A, Uciechowski P, Floege J, von der Ohe J, Resch K, Radeke HH: Autocrine growth regulation of human glomerular mesangial cells is primarily mediated by basic fibroblast growth factor. *Am J Pathol* 147: 1372–1382, 1995
  51. Ray PE, Liu XH, Xu L, Rakusan T: Basic fibroblast growth factor in HIV-associated hemolytic uremic syndrome. *Pediatr Nephrol* 13: 586–593, 1999
  52. Parmar MK, Machin D: *Survival Analysis: A Practical Approach*. Chichester, John Wiley and Son Ltd., 1995
  53. Cross MJ, Claesson-Welsh L: FGF and VEGF function in angiogenesis: Signalling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol Sci* 22: 201–207, 2001
  54. Suga SI, Kim YG, Joly A, Puchacz E, Kang DH, Jefferson JA, Abraham JA, Hughes J, Johnson RJ, Schreiner GF: Vascular endothelial growth factor (VEGF121) protects rats from renal infarction in thrombotic microangiopathy. *Kidney Int* 60: 1297–1308, 2001
  55. Kim YG, Suga SI, Kang DH, Jefferson JA, Mazzali M, Gordon KL, Matsui K, Breiteneder-Geleff S, Shankland SJ, Hughes J, Kerjaschki D, Schreiner GF, Johnson RJ: Vascular endothelial growth factor accelerates renal recovery in experimental thrombotic microangiopathy. *Kidney Int* 58: 2390–2399, 2000
  56. Floege J, Eng E, Young BA, Alpers CE, Barrett TB, Bowen-Pope DF, Johnson RJ: Infusion of platelet-derived growth factor or basic fibroblast growth factor induces selective glomerular mesangial cell proliferation and matrix accumulation in rats. *J Clin Invest* 92: 2952–2962, 1993
  57. Zhang GH, Ichimura T, Wallin A, Kan M, Stevens JL: Regulation of rat proximal tubule epithelial cell growth by fibroblast growth factors, insulin-like growth factor-1 and transforming growth factor-beta, and analysis of fibroblast growth factors in rat kidney. *J Cell Physiol* 148: 295–305, 1991
  58. Floege J, Burg M, Hugo C, Gordon KL, Van Goor H, Reidy M, Couser WG, Koch KM, Johnson RJ: Endogenous fibroblast growth factor-2 mediates cytotoxicity in experimental mesangio-proliferative glomerulonephritis. *J Am Soc Nephrol* 9: 792–801, 1998
  59. Floege J, Eng E, Lindner V, CEA, Young BA, Reidy MA, Johnson RJ: Rat glomerular mesangial cells synthesize basic fibroblast growth factor. Release, upregulated synthesis, and mitogenicity in mesangial proliferative glomerulonephritis. *J Clin Invest* 90: 2362–2369, 1992
  60. Floege J, Kriz W, Schulze M, Susani M, Kerjaschki D, Mooney A, Couser WG, Koch KM: Basic fibroblast growth factor augments podocyte injury and induces glomerulosclerosis in rats with experimental membranous nephropathy. *J Clin Invest* 96: 2809–2819, 1995

See related article, “Basic Fibroblast Growth Factor among Children with Diarrhea-Associated Hemolytic Uremic Syndrome,” on pages 699–707.