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

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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receptor antagonist (4), transforming growth factor β-1 (5), platelet activating factor (Smith JM, *et al.*, unpublished data), degraded von Willebrand factor multimers (6), and plasma factors that demonstrate thrombin generation, intravascular fibrin accretion, and fibrinolysis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 β_2-microglobulin and *N*-acetyl-β-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).

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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.

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