Diarrhea-associated hemolytic uremic syndrome (D+HUS) is the most common cause of acute renal failure among previously healthy children in the United States (1). Most cases are caused by antecedent infections with Shiga toxin (Stx)-producing Escherichia coli. Stx bind to a glycolipid receptor on the endothelial cell surface, leading to diffuse vascular injury and organ failure (1,2).

Cytokines play an important role in enhancing the cytotoxic effects of Stx (2). A number of inflammatory cytokines, including interleukin-1α (IL-1α), IL-6, IL-8, and tumor necrosis factor-α (TNF-α), have been implicated in the pathogenesis of D+HUS (3–5). These cytokines may reflect the systemic response of the host to Stx or other bacterial products. Alternatively, because endothelial injury is the primary pathogenic event in D+HUS, other cytokines that are associated with endothelial cells and released after vascular damage, such as basic fibroblast growth factor (bFGF), may play a role in this disease. High levels of bFGF in the circulation and renal tissue were documented for a small group of children affected by severe clinical forms of typical or HIV-related hemolytic uremic syndrome (HUS) (6,7).

bFGF is an 18-kD, cationic, heparin-binding peptide that is released after endothelial cell injury. It promotes angiogenesis, neovascularization, wound healing, and renal growth (6). bFGF lacks a classic signal peptide sequence for extracellular export and is not secreted in a conventional manner by producer cells. Instead, it is primarily a cell-associated protein that is normally stored as an “inactive pool” in the walls of blood vessels. bFGF is deposited in the extracellular matrix of most samples more frequently than were IL-1α, IL-8, and tumor necrosis factor-α. There was an acute increase in urinary bFGF excretion, which returned to normal during convalescence. Urinary excretion of bFGF during the acute phase was higher among patients who required dialysis, compared with those who did not (48.9 ± 15.0 and 28.9 ± 9.0 pg/ml, respectively; P < 0.05). Plasma bFGF concentrations were persistently elevated throughout the period of hospitalization and the follow-up period among patients with D+HUS. Urinary excretion and plasma levels of bFGF were comparable for the SYNSORB Pk-treated (n = 19) and placebo-treated (n = 12) groups. Measurements of urinary and plasma concentrations of bFGF among patients with D+HUS may be useful indices for assessment of the severity of acute renal disease and the timing and adequacy of the systemic angiogenic process during early convalescence.

Abstract. Diarrhea-associated hemolytic uremic syndrome (D+HUS) is characterized by endothelial injury and activation of inflammatory cytokines. Basic fibroblast growth factor (bFGF) is an angiogenic peptide released in response to vascular damage. The plasma concentrations and urinary excretion of bFGF during the course of D+HUS were determined, in comparison with the levels of various inflammatory cytokines, and changes were correlated with clinical and laboratory features of the disease. Serial plasma and urine samples were collected from 31 children with D+HUS, during the acute (days 1 to 7 of hospitalization) and recovery (through day 60 after discharge from the hospital) phases of the disease. The patients were enrolled in the multicenter trial of SYNSORB Pk (SYNSORB Biotech, Calgary, Alberta, Canada) treatment for D+HUS. bFGF, interleukin-1α (IL-1α), IL-8, and tumor necrosis factor-α levels were determined with enzyme-linked immunosorbent assays. bFGF was detected in urine and plasma
organs, including the kidney (6,7). bFGF is not normally present in the circulation unless it is released via nonconventional pathways during angiogenesis, tumor growth, and/or vascular injury (6,8,9). In view of the diffuse endothelial injury in patients with D+HUS, it might be expected that there would be alterations in the circulating levels and urinary excretion of bFGF attributable to release of the peptide by damaged endothelial and renal tubular epithelial cells. However, bFGF levels have not been systematically examined in a large sample of children with D+HUS. Moreover, previous studies did not correlate these changes with levels of other cytokines or relevant clinical or laboratory indices of disease severity. Therefore, we performed these studies with a randomly selected subgroup of children who were enrolled in a trial of SYNSORB Pk (SYNSORB Biotech, Calgary, Alberta, Canada) treatment for D+HUS, with two specific aims, i.e., to determine the serial changes in the plasma concentrations and urinary excretion of bFGF, in comparison with IL-1α, IL-8, and TNF-α, and to correlate alterations in bFGF and inflammatory cytokine production with renal function and other clinical and laboratory features among children with D+HUS.

**Materials and Methods**

**Subjects**

The study was approved by the institutional review boards of Schneider Children’s Hospital of the North Shore-Long Island Jewish Health System and the participating sites in the multicenter trial. Informed consent for treatment with study medication and for the use of stored samples in future studies was obtained before enrollment into the clinical trial.

Children between the ages of 6 mo and 18 yr with D+HUS, who were enrolled in a multicenter, randomized, placebo-controlled, clinical trial of SYNSORB Pk, were eligible for inclusion in this study. The diagnosis of D+HUS was made on the basis of the following four criteria: (1) platelet count of <140,000/mm³; (2) fragmentation of erythrocytes evident in a peripheral smear; (3) renal injury, as indicated by the presence of hematuria, proteinuria, and/or azotemia; and (4) a diarrheal illness within 7 d of the identification of HUS. Exclusion criteria included (1) an atypical nonidiopathic prodrome; (2) a family history of hereditary HUS; (3) HUS associated with bone marrow transplantation, pneumococcal infection, or HIV infection; (4) preexisting renal disease; and (5) preexisting structural abnormalities or motility disorder of the gastrointestinal tract.

An operational diagnosis of D+HUS, based on a prodromal diarrheal illness, was adopted to expedite patient enrollment and initiation of study medication. Extensive microbiologic investigations were performed for all study patients. Eighteen of 31 of the children (58%) described in this report exhibited evidence of Stx-producing *E. coli* infections (three with Stx1-producing strains, eight with Stx2-producing strains, and seven with combined Stx1/2-producing microorganisms).

Control subjects (*n* = 17) were recruited at two sites, i.e., children who were being regularly monitored in the nephrology clinic at Schneider Children’s Hospital (*n* = 7) and patients who were admitted to Children’s National Medical Center for treatment of acute nonrenal diseases (*n* = 10). The outpatients exhibited a variety of conditions, including renal transplants (*n* = 2), minimal-change nephrotic syndrome, focal segmental glomerulosclerosis, IgA nephropathy, nonspecific glomerulonephritis, and X-linked hypophosphatemia. All of the control subjects exhibited normal kidney function. Urine and plasma samples were obtained from these control patients.

**SYNSORB Pk Study Design**

Patients with D+HUS were randomly assigned to receive SYNSORB Pk or cornmeal placebo, in a 2:1 ratio. On the basis of an analysis of the outcomes for 120 patients (69% of the target sample of 174 children), the Data Safety Monitoring Board concluded that SYNSORB Pk had no effect on the frequency of death or serious extrarenal events or the need for dialysis support among children with D+HUS (the two primary end points of the trial). Therefore, the clinical trial was terminated on April 17, 2001, after enrollment of 150 patients.

Demographic information was compiled for all patients, including data on age, gender, and race. In addition, the following clinical features were noted: length of hospitalization, need for dialysis, and occurrence of extrarenal events or death. The decision to initiate dialysis was based on 72 h consecutively of oligoanuria, i.e., <0.5 ml/kg/h, in an attempt to standardize the use of this treatment modality. Renal biopsies were not routinely performed because the procedure is rarely indicated for patients with D+HUS (10). Select laboratory data, i.e., peak blood urea nitrogen (BUN) and creatinine concentrations, highest white blood cell count, and lowest platelet count, were recorded, together with the day of occurrence of each abnormality. The presence of proteinuria or hypertension and the creatinine clearance were assessed at the end of the 60-d follow-up period after discharge from the hospital.

**Study Protocol**

Plasma samples were to be collected on days 1, 4, 7, and 10 of hospitalization and day 28 after discharge from the hospital. Urine and stool samples were to be collected daily during the first 7 d of hospitalization, on day 10 of hospitalization, and on days 7, 14, 28, and 60 after discharge from the hospital. The concentrations of IL-1α, IL-8, and TNF-α were determined in all three body fluids, and residual samples were stored at −70°C. Plasma and urine samples from 31 children were selected for determination of bFGF levels. These patients, who were enrolled in the study from its onset on July 27, 1997, through September 4, 1998, were randomly chosen because they were the first subjects for whom all cytokine assays required by the protocol were completed and for whom residual samples were available.

**Immunoassays**

Plasma and urinary cytokine (IL-1α, IL-8, and TNF-α) concentrations were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Endogen, Cambridge, MA). The lower limits for detection of IL-1α, IL-8, and TNF-α in urine and plasma samples were 10.25, 25.6, and 25.6 pg/ml, respectively. Plasma and urinary levels of bFGF were measured with two ELISA of different sensitivities (R&D Systems, Minneapolis, MN), as indicated by the instructions provided by the manufacturer (ranges of 0.5 to 64 and 10 to 640 pg/ml for plasma and urine assays, respectively).

**Western Blots**

The presence of bFGF in urine samples was confirmed by Western blot analysis using the ECL Western blotting kit from Amersham (Life Science, Buckinghamshire, UK), as described previously (7). Briefly, urine samples were cleared by centrifugation and loaded onto 12.5% sodium dodecyl sulfate-polyacrylamide gels. The electro-
phoretically separated proteins were transferred to nitrocellulose filters by electroblotting and were immunostained with specific, affinity-purified, polyclonal IgG antibodies against a unique peptide sequence of bFGF (1:2000 dilution; generously provided by Dr. Andrew Baird, Przim Pharmaceuticals, San Diego CA) (7,11).

Statistical Analyses
Samples in which the cytokine or bFGF levels were undetectable were assigned arbitrary values equal to the lowest standard value in the particular assay. Urinary bFGF and cytokine levels were normalized per milligram of creatinine, because the specimens were spot samples and not timed collections. For some patients, not all plasma and urine samples were obtained in accordance with the protocol. Therefore, to simplify the analysis and interpretation of the data, the mean bFGF and cytokine concentrations in the plasma and urine for each patient were calculated for two nonoverlapping time periods, i.e., the acute phase (days 1 to 7 after entry into the SYNSORB Pk study) and the recovery phase (day 10 of hospitalization until the end of the follow-up period). The specimens available for these two phases were not pooled into a single sample. Instead, mean values for urinary excretion and plasma bFGF levels were calculated for each patient, on the basis of the available measurements for the acute and recovery periods. Data are expressed as mean ± SEM. Differences with time and between the D+HUS group and the control group were analyzed with an ANOVA and t test. Differences in proportions between groups were analyzed with the χ² test. Linear regression analyses were performed to determine the correlation between plasma and urinary bFGF levels and levels of the other cytokines, as well as the clinical and laboratory variables listed above. The results were considered significant at P < 0.05.

Results
Clinical Features
The mean age of the 31 patients enrolled in the SYNSORB Pk clinical trial who were included in this study was 5.2 ± 0.6 yr (range, 1.3 to 15.3 yr), the male/female ratio was 8:23, and the body weight was 21.5 ± 2.4 kg. There were 26 white children, four Hispanic children, and one Asian child. The length of the hospital stay was 10.9 ± 1.3 d. There were no deaths, but two of 31 patients (6.5%) experienced extrarenal complications, namely respiratory failure in one child and respiratory failure, seizures, and diabetes mellitus in a second patient. Dialysis was used to treat acute renal failure in nine of 31 cases (29%). The mean age, the incidence of serious extra-renal events, and the need for dialysis were comparable for this patient cohort versus the entire sample.

The most abnormal laboratory values and their timing are summarized in Table 1. The results were similar to the data compiled for the full patient sample. It is evident that nearly all of the indices of disease severity were most significantly deranged on days 3 to 4 of the illness. The platelet count nadir occurred approximately 1 d before the maximal disturbances in kidney function, leukocytosis, and anemia (P < 0.01, compared with the time of the lowest hematocrit values).

The 60-d follow-up evaluation occurred 71 ± 4 d after entry into the study. At that time, only one of 19 children for whom an early morning urine specimen was available exhibited proteinuria. A 40-yr-old boy exhibited mild systolic hypertension (BP, 128/65 mmHg). Seventeen children were old enough to undergo determination of GFR in creatinine clearance assays, and the mean value was 141 ± 26 ml/min per 1.73 m². The creatinine clearance was <90 ml/min per 1.73 m² (33, 43, 48, 68, and 80 ml/min per 1.73 m²) in five cases.

The 17 control patients (nine male patients) were monitored in the nephrology clinic. Their mean age was 6.8 ± 1.5 yr (range, 1.0 to 20.5 yr). There were 10 white children, three Hispanic children, and four black children. The mean ages, gender distributions, and ethnicity were comparable for the patients with D+HUS and the control subjects. The GFR was normal for all patients, and the mean serum creatinine level was 0.61 ± 0.10 mg/dl. At the time of sample collection, none of the patients exhibited diarrhea or other gastrointestinal complaints.

Table 1. Clinical features of children with D+HUS (n = 31)a

<table>
<thead>
<tr>
<th></th>
<th>Peak Value</th>
<th>Day of Occurrence</th>
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<tbody>
<tr>
<td>BUN level (mg/dl)</td>
<td>77 ± 39</td>
<td>3.6 ± 4.0</td>
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<tr>
<td>Creatinine level (mg/dl)</td>
<td>3.5 ± 2.7</td>
<td>3.5 ± 3.5</td>
</tr>
<tr>
<td>WBC count (× 10⁹/L)</td>
<td>19.5 ± 10.7</td>
<td>3.7 ± 3.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>18.5 ± 3.0</td>
<td>3.5 ± 1.7</td>
</tr>
<tr>
<td>Platelet count (× 10⁹/L)</td>
<td>32.7 ± 33.2</td>
<td>2.4 ± 1.4b</td>
</tr>
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</table>

a D+HUS, diarrhea-associated hemolytic uremic syndrome; BUN, blood urea nitrogen; WBC, white blood cell. Data are provided as mean ± SEM.

b P < 0.01 versus day of occurrence of lowest hematocrit value.

Urinary and Plasma bFGF Concentrations
Among children with D+HUS, bFGF was detected in the urine in 24 of 31 cases (77%) during the initial 7 d of disease. There was a significant increase in the urinary excretion of bFGF during the first 7 d of the illness, compared with the control subjects. During the recovery phase, urinary bFGF levels were below the detectable limit in 14 of 28 cases, and the mean value returned to normal at that stage of the illness (Figure 1). The proportion of urine samples with detectable bFGF levels was significantly higher during the acute phase, compared with the recovery phase (P < 0.04). Throughout the entire course of D+HUS, only two children exhibited undetectable plasma bFGF levels, one during the acute phase and the second during convalescence. In contrast to the inverted V-shaped pattern observed for urinary bFGF excretion, the mean plasma concentrations of bFGF were elevated during the acute phase and remained significantly higher than those for the control subjects through day 28 of the follow-up period after discharge from the hospital (Figure 2). The urinary excretion and plasma concentrations of bFGF were comparable for the children who received SYNSORB Pk (n = 19) and those who received placebo (n = 12) (Table 2).

The individual values for urinary (Figure 3A) and plasma (Figure 3B) levels of bFGF during the two phases of D+HUS are provided in scatter plots. Evaluations of serial changes in urinary bFGF excretion for individual patients indicated that the amount increased during the recovery period, compared
with the acute phase, in 14% of the cases, decreased by <75% in an additional 14% of the cases, and decreased by >75% in the remaining 72% of the cases. During the course of the follow-up period, the plasma bFGF levels increased by >25% in 48% of the cases, remained the same in 10% of the cases, and decreased by >25% in 42% of the cases. The bFGF detected with the ELISA kits in the urine of children with D+HUS was the intact molecule, as confirmed by immunoblot analysis (Figure 4).

### IL-1α, IL-8, and TNF-α Levels

There was a great deal of variability in IL-1α, IL-8, and TNF-α levels during the course of D+HUS. TNF-α levels were below the limit of detection for all except five urine samples, four (13%) obtained from children during the acute phase and one (3%) obtained from a patient during recovery. Only three patients exhibited measurable plasma TNF-α levels, two (7%) during the first 7 d of the illness and one (3%) during convalescence. Similarly, four other children with D+HUS exhibited detectable plasma IL-1α concentrations, three (10%) during the first 7 d of the illness and one (3%) during convalescence. IL-1α was detectable in the urine of 12 children (39%) during the acute phase and 10 (32%) during recovery (Figure 5A). Interestingly, six patients exhibited detectable IL-1α in urine samples collected during both the acute and convalescence phases; the levels decreased with time for five of those children. Urine samples from 20 children (65%) during the acute phase and 15 children (48%) during convalescence exhibited detectable IL-8 levels (Figure 5B). In plasma samples, IL-8 was detectable for 23 children (74%) during the first 7 d of the illness but for only five children (16%) during the convalescence period (Figure 6). The levels of the three inflammatory cytokines and bFGF in urine and plasma samples obtained from the control patients are summarized in Table 3.

### Correlations between bFGF and Cytokine Concentrations and Clinical Features and Levels of Other Cytokines

There was a trend toward positive correlations between the urinary excretion of bFGF during the acute phase of D+HUS and the peak BUN ($r = 0.32, P = 0.096$) and creatinine ($r = 0.30, P = 0.11$) concentrations. Moreover, urinary excretion of bFGF was higher among the children who required dialysis, compared with those who did not require acute renal replacement therapy ($48.9 ± 15.0$ and $28.9 ± 9.0$ pg/ml, respectively;
In addition, the relationship between urinary bFGF excretion during days 1 to 7 of the illness and the platelet count nadir was of borderline significance ($r = 0.36$, $P = 0.05$). There was no correlation between urinary bFGF excretion and the urinary concentrations of the other three cytokines during the first 7 days of D+HUS. The plasma bFGF concentration during the acute phase was highly correlated with the platelet count nadir ($r = 0.67$, $P < 0.0001$) and the plasma IL-8 concentration during the acute phase of the disease ($r = 0.61$, $P = 0.0003$). The plasma concentrations of bFGF were similar for the children who required dialysis and those who did not require that treatment ($15.7 \pm 6.9$ and $15.2 \pm 3.8$ pg/ml, respectively). Finally, neither urinary bFGF excretion nor plasma bFGF levels were correlated with the peak circulating white blood cell count ($P > 0.6$).

There was no relationship between urinary excretion or plasma concentrations of bFGF and any of the clinical features or cytokine levels during the recovery phase after treatment of D+HUS. In comparison with bFGF, the levels of IL-1$\alpha$, IL-8, and TNF-$\alpha$ in plasma or urine specimens from the children with D+HUS were not correlated with any measure of renal function during the acute or recovery phase of the disease. A correlation between the urinary concentrations of IL-1$\alpha$ and IL-8 and the peak white blood cell count during the first 7 days of D+HUS were the only significant relationships noted for these three cytokines.

**Discussion**

Our results indicate that, among children with D+HUS, there is a significant increase in the urinary excretion of bFGF during the acute phase of the disease, which returns to normal during convalescence. In contrast, a pronounced increase in the plasma levels of bFGF persists during the first 4 wk after discharge from the hospital. The high levels of urinary excretion of bFGF during the acute phase of D+HUS, when GFR is profoundly reduced, suggest that the peptide is primarily of renal origin at that stage of the illness. The elevated plasma bFGF concentrations after improvements in kidney function are consistent with increased systemic production of the growth factor, rather than impaired renal clearance. In general, during the course of D+HUS, bFGF was detectable in urine and plasma samples more often than IL-1$\alpha$, IL-8, and TNF-$\alpha$.

Only two previous studies examined the levels of bFGF in HUS. In the first, very high levels of bFGF were documented in urine and plasma samples obtained from 10 children who died or experienced severe D+HUS (6). Ray et al. (8) described two children with HIV-associated HUS for whom plasma bFGF concentrations were elevated, compared with children with HIV nephropathy or HIV infection without renal disease. Immunohistochemical studies revealed increased levels of bFGF in renal sections from children with severe D+HUS or HIV-related HUS, compared with kidney tissue from children with other renal diseases. bFGF was localized to heparan sulfate proteoglycans in glomeruli and the tubulointerstitium. bFGF isolated from the urine was biologically active.
and induced mitogenic/regenerative changes in primary cultures of glomerular or tubular cells. On the basis of these findings, it was suggested that, in D+HUS, bFGF that is released into the circulation by injured endothelial cells is trapped in the kidney and modulates the repair and regeneration of damaged renal vascular and epithelial cells. Serial measurements of the plasma concentrations and urinary excretion of bFGF were not performed in either study. Our data represent the first systematic, large-scale investigation of bFGF levels during the course of D+HUS.

bFGF lacks a signal peptide for secretion and is not exported from cells by conventional pathways. Under normal circumstances, there are very low levels of bFGF in the circulation (8). Previous studies demonstrated that bFGF is released by injured renal endothelial and tubular cells (12,13). Renal epithelial cells are sensitive to the cytotoxic action of Stx (14–17). Therefore, Stx may induce the release of bFGF by injured endothelial and renal tubular cells in D+HUS.

Because the direct effects of Stx alone cannot explain the pathogenesis and histologic lesions of D+HUS, there is a great deal of interest in the roles of cytokines and chemokines in this disease. Elevated plasma levels and increased urinary excretion of a wide variety of inflammatory mediators, such as IL-1β, IL-6, IL-8, TNF-α, and monocyte chemotactic protein-1, have been demonstrated during the acute phase of D+HUS (3–5,18,19). It has been suggested that serial measurements of specific cytokines, such as IL-6 and IL-8, can be used to monitor disease activity in D+HUS (3,20). In the multicenter clinical trial of SYNSORB Pk, levels of three cytokines, i.e., IL-1α, IL-8, and TNF-α, were serially measured in plasma and urine samples from all patients. These measurements allowed the sensitivity and utility of these inflammatory cytokines as markers of the severity and resolution of D+HUS to be compared with those of bFGF.

Larger percentages of urine and plasma samples tested positive for bFGF, compared with IL-1α, IL-8, and TNF-α, throughout the course of D+HUS. Our data regarding the inflammatory cytokines in D+HUS are in general agreement with previously published reports (21). The infrequent detection of TNF-α among our patients is consistent with the results of studies by Fitzpatrick et al. (20), Karpman et al. (3), and Proulx et al. (22), in which TNF-α could be measured in the plasma of only one of 16, seven of 35, and none of 28 children with D+HUS, respectively. The difference between our findings and those of Lopez et al. (23) may reflect differences in the microbiologic causes of D+HUS in the United States versus Argentina (24), the severity of the disease itself, the timing of sampling, or the assay procedures. Of note, Lopez et

Figure 5. Scatter plots of urinary cytokine levels during the acute and recovery phases of D+HUS. The values for the acute and recovery phases were calculated by determining the average value for all available specimens for the two nonoverlapping time periods for each patient and then determining the overall mean for each phase of the study. The bars represent the mean values for the patients with D+HUS during the recovery period. The mean urinary levels of interleukin-1α (IL-1α) and IL-8 during the acute phase were 3692 and 509,999 pg/mg creatinine, respectively. The numbers above the columns of open circles represent cytokine values outside the indicated concentration range. (A) IL-1α. (B) IL-8.

Figure 6. Scatter plot of plasma IL-8 concentrations during the acute and recovery phases of D+HUS. The values for the acute and recovery phases were calculated by determining the average value for all available specimens for the two nonoverlapping time periods for each patient and then determining the overall mean for each phase of the study. The bars represent the mean values for each group. The number above the column of open circles represents an IL-8 value outside the indicated concentration range.
The correlations noted between bFGF levels and select laboratory measurements, such as the degree of azotemia and thrombocytopenia, were stronger than the relationships observed for the inflammatory cytokines. Therefore, bFGF may be a more valuable marker for assessment of the severity of D+HUS (especially the renal component) during the acute phase. Release of bFGF, compared with the inflammatory cytokines, may be less affected by other clinical features, e.g., fever or dehydration.

The abnormalities in urinary excretion and plasma concentrations of bFGF during D+HUS may reflect two distinct processes. In the acute phase, there is both systemic vascular injury and intrarenal endothelial and tubular damage provoked by Stx (9,27,28). This causes release of bFGF into the circulation, leakage into the urine, and an increase in the urinary excretion of bFGF. However, the stronger correlation between urinary bFGF excretion, compared with plasma bFGF levels, and the indices of renal dysfunction (e.g., peak BUN and creatinine levels and the need for dialysis) suggests that acute changes in urinary bFGF levels are determined primarily by the extent of renal injury. Normalization of urinary bFGF excretion during the 60-d follow-up period may indicate the cessation of vascular and epithelial injury within the kidney. In contrast, the prolonged increase in plasma bFGF levels during recovery from D+HUS, even after improvements in renal function and normalization of urinary bFGF excretion, may be a marker of ongoing angiogenesis to restore organ perfusion (29). Differential damage of endothelial versus tubular cells by Stx and the systemic nature of this angiogenic response may explain why circulating bFGF levels were similar regardless of whether patients required dialysis. This hypothesis is consistent with the capacity of bFGF to promote endothelial cell growth and to modulate the activity of proteases and the plasminogen-fibrinolysis cascade involved in the regeneration of new blood vessels (29,30). Determination of peak urinary bFGF excretion and the time to normalization of plasma bFGF levels, in combination with other clinical measurements (such as assessments of proteinuria and BP), may facilitate predictions of the risk of permanent damage to the kidney and other organs after an episode of D+HUS.

There was no significant difference in the urinary excretion or plasma concentrations of bFGF during the observation period for children who received SYNISORB Pk versus placebo. This is consistent with the lack of any effect of this experimental treatment on the course of D+HUS. It remains to be determined whether novel therapies that attenuate the severity of this disease could alter the pattern of bFGF metabolism described in this report.

In summary, we demonstrated that children with D+HUS exhibited high urinary and plasma levels of bFGF during the acute stages of the disease. Although urinary excretion of bFGF returned to normal during recovery, the plasma concentrations of the peptide remained high during a 60-d convalescence period. bFGF was detected in urine and plasma samples more frequently than were three inflammatory cytokines, namely IL-1α, IL-8, and TNF-α. During the acute phase of D+HUS, both the urinary and plasma levels of bFGF were inversely related to the lowest platelet count, and the plasma bFGF concentrations were correlated with the circulating levels of IL-8. Urinary excretion of bFGF, but not the plasma concentrations of the peptide, was significantly higher among children who required dialysis, suggesting increased renal production and/or release of bFGF in the most severely injured kidneys. We speculate that bFGF is involved in the pathogenesis of and recovery from D+HUS. Plasma and urinary concentrations of bFGF among children with D+HUS may be useful indices for assessment of the severity of acute renal damage and short-term clinical outcomes after this illness.

### Table 3. Levels of bFGF and inflammatory cytokines among control subjects

<table>
<thead>
<tr>
<th></th>
<th>bFGF</th>
<th>IL-1α</th>
<th>IL-8</th>
<th>TNF-α</th>
</tr>
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<tbody>
<tr>
<td><strong>Plasma level (pg/ml)</strong></td>
<td>3.3 ± 0.6 (17)</td>
<td>77.9 ± 25.7 (7)</td>
<td>453 ± 364 (7)</td>
<td>&lt;25.6 (7)</td>
</tr>
<tr>
<td><strong>Urinary excretion (pg/ml creatinine)</strong></td>
<td>3.6 ± 1.0 (17)</td>
<td>53.3 ± 14.9 (7)</td>
<td>540 ± 490 (7)</td>
<td>19.1 ± 4.2 (7)</td>
</tr>
</tbody>
</table>

* IL, interleukin; TNF, tumor necrosis factor. Data are provided as mean ± SEM. The numbers in parentheses indicate the number of samples assayed for each specific cytokine.
Appendix: Participating Centers

Administrative center: Howard Trachtman, M.D. (principal investigator), and Erica Christen, R.N. (project coordinator), Schneider Children’s Hospital (New Hyde Park, NY); data-coordinating center: Avital Cnaan, Ph.D., and Kathleen Gibbs, M.S., Children’s Hospital of Philadelphia (Philadelphia, PA); microbiology core laboratory: David Acheson, M.D., and Ramona Chitrakar, Tufts University-New England Medical Center (Boston, MA); data safety monitoring board: Julie Ingelfinger, M.D., Gladys Hirschman, M.D., Josephine Briggs, M.D., John Kusek, M.D., Daniel Cattran, M.D., Mitchell B. Cohen, M.D., Katherine Freeman, Ph.D., Thomas Greene, Ph.D., and Solomon Moshe, M.D.; participating centers: Howard Trachtman, M.D., Schneider Children’s Hospital (New Hyde Park, NY); Seth Schulman, M.D., Children’s Hospital of Philadelphia (Philadelphia, PA); James Springate, M.D., Children’s Hospital of Buffalo (Buffalo, NY); Frederick Kaskel, M.D., Ph.D., Montefiore Medical Center (Bronx, NY); Dilysh Whyte, M.D., State University of New York Hospital at Stony Brook (Stony Brook, NY); Robert Weiss, M.D., New York Medical College/Westchester County Medical Center (Valhalla, NY); Charles McKay, M.D., du Pont Hospital for Children (Wilmington, DE); Lewis Reisman, M.D., St. Barnabas Hospital for Children (Livingston, NJ); Eduardo Perelstein, M.D., Cornell University Medical Center (New York, NY); Manju Chandra, M.D., North Shore University Hospital (Manhasset, NY); Jose Salcedo, M.D., St. Joseph’s Children’s Hospital ( Patterson, NJ); Lynne Weiss, M.D., New York Medical College/Westchester County Medical Center (New Brunswick, NJ); William Varade, M.D., State University of New York Rochester Medical Center (Rochester, NY); Douglas Ford, M.D., Denver Children’s Hospital (Denver, CO); James Chan, M.D., Medical College of Virginia (Richmond, VA); Irene Restaino, M.D., Children’s Hospital of the King’s Daughters (Norfolk, VA); Shashi Nagaraj, M.D., Wake Forest University/North Carolina Baptist Hospital (Winston-Salem, NC); Victoria Norwood, M.D., University of Virginia Medical Center (Charlottesville, VA); John Foreman, M.D., Duke University Medical Center (Durham, NC); Michael Moritz, M.D., Children’s Hospital of Pittsburgh (Pittsburgh, PA); John Mahan, M.D., Columbus Children’s Hospital (Columbus, OH); Marva Moxey-Mims, M.D., Children’s National Medical Center (Washington DC); Barry Warshaw, M.D., Egleston Children’s Hospital (Atlanta, GA); Verna Yiu, M.D., University of Alberta Hospital (Edmonton, Alberta, Canada); Andrew Brem, M.D., Rhode Island Hospital (Providence, RI); Sharon Bartosh, M.D., University of Wisconsin Hospital (Madison, WI); Sharon Andreoli, M.D., University of Indiana/Riley Children’s Hospital (Indianapolis, IN); Lawrence Milner, M.D., Tufts University-New England Medical Center (Boston, MA); Jens Goebel, M.D., University of Kentucky Medical Center (Lexington, KY); Dianne Muchant, M.D., West Virginia University Medical Center (Morgantown, WV); and Coral Hanevold, M.D., Medical College of Georgia (Augusta, GA).

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