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Basic fibroblast growth factor in HIV-associated hemolytic uremic syndrome

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Abstract Endothelial injury is the primary pathogenic event leading to the renal thrombotic microangiopathic lesions typical of the hemolytic uremic syndrome (HUS). Basic fibroblast growth factor (bFGF) is an angiogenic growth factor released by injured endothelial cells. In a previous study we have found a significant accumulation of bFGF in human immunodeficiency virus (HIV)-transgenic mice with renal disease. Here we investigated whether bFGF was accumulated in the circulation and kidneys of two children with HIV-associated HUS (HIV-HUS), and studied the mechanisms involved in this process. The plasma levels of bFGF in children with HIV-HUS (124±20 pg/ml) were increased compared with five children with HIV nephropathy (49±6 pg/ml) and twenty HIV-infected children without renal disease (26±4 pg/ml, $P<0.001$). Immunohistochemistry and receptor binding studies showed that bFGF was accumulated bound to heparan sulfate proteoglycans in renal glomeruli and interstitium surrounding renal tubules in HIV-HUS kidneys. Basic FGF stimulated the proliferation of mesangial and urinary renal tubular epithelial cells isolated from both patients. These findings support the hypothesis that bFGF and its low-affinity binding sites may play a relevant role in modulating the process of glomerular and renal tubular regeneration during the acute stages of HIV-HUS. A follow-up study in a larger sample population is required to confirm these results.

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Introduction

The hemolytic uremic syndrome (HUS) is an important cause of renal failure in children, characterized by thrombocytopenia, hemolytic anemia, and acute renal failure [1, 2]. Classic (D+) or epidemic HUS is primarily associated with a diarrhea syndrome caused by bacteria producing Shiga-like toxins in young children [3, 4], while atypical or sporadic HUS is associated with multiple factors, including infectious agents, malignancies, drugs, and systemic diseases affecting older children or adults [4–7].

Several studies provide evidence for a chronic state of endothelial injury in human immunodeficiency virus (HIV)-infected patients [8–11]. Thus, it is possible that vascular growth factors released by injured endothelial cells may be released in the circulation of HIV-infected children. In a previous study we found a significant accumulation of basic fibroblast growth factor (bFGF) in the kidneys of HIV- transgenic mice with renal disease [12]. bFGF is an angiogenic vascular growth factor that is normally stored as an "inactive pool" in the vessel wall and extracellular matrix, and is not present in the circulation unless it is released during angiogenesis, tumor growth, and/or vascular injury [13–15]. Since bFGF is released by injured endothelial cells and peripheral blood mononuclear cells [16], and is involved in the pathogenesis of HIV nephropathy in transgenic mice [12], we investigated changes in bFGF release and activity in two children who developed HIV-HUS.

We found a significant accumulation of biologically active bFGF in the plasma, urine, and kidneys of these children with HIV-HUS. In the kidney, bFGF was accumulated bound to an increased number of heparan sulfate proteoglycans (HSPG), in close association with proliferating and regenerating renal tubular epithelial cells

(RTEc). Moreover, mesangial cells and RTEc isolated from these patients responded to bFGF mitogenic effects. Thus, when these results are interpreted in the context of previous studies [12, 17, 18], they support the hypothesis that bFGF is released into the circulation of children with HIV-HUS and stored bound to an increased number of renal bFGF low-affinity receptors leading to the proliferation of renal cells.

Patients and methods

Patients

This study was approved by the Institutional Review Board of Children's National Medical Center in Washington D.C. We studied two HIV-infected children who developed HIV-HUS. The epidemiology, diagnosis, treatment, and clinical outcome of both patients have been described in detail in a previous publication [7]. The diagnosis of HIV-HUS in both patients was confirmed clinically and histologically [7]. Both patients developed a clinical course characterized by severe renal tubular injury and progressive downhill course leading to end-stage renal disease and death.

Patient 1 was a 12-year-old HIV-positive white male who acquired HIV-1 through a blood transfusion at age 3 years. At the time of admission, his blood urea nitrogen was 23 mg/dl and serum creatinine 1.5 mg/dl. His hematocrit was 19%, hemoglobin 6.5 g/dl, white blood count 2,200/µl platelet count 120,000/µl, reticulocyte count 8.6%, haptoglobin <10 mg/dl, and fibrinogen 323 mg/dl. Cultures of his blood, urine, and stools were negative. The peripheral smear showed evidence of microangiopathic hemolytic anemia. His prothrombin time was 10.9 s, partial thromboplastin time 32.4 s, and fibrin degradation products were negative. His urinalysis revealed a pH of 8.5, specific gravity of 1.008, 4+ protein, 3+ blood, and several granular casts. On renal ultrasonography both kidneys were echogenic. A renal biopsy was performed when his creatinine rose to 2.6 mg/dl and hemodialysis was initiated when his serum creatinine reached 7.3 mg/dl.

Patient 2 was a 6-month-old African-American female with HIV encephalopathy who acquired HIV-1 through vertical transmission. At the time of admission, her blood urea nitrogen was 8 mg/dl and serum creatinine 0.6 mg/dl. Her hematocrit was 20%, hemoglobin 6.4 g/dl, white blood count 6,000/µl, platelet count 58,000/µl, reticulocytes count 2.5%, and haptoglobin <10 mg/dl. The peripheral smear showed evidence of microangiopathic hemolytic anemia. Prothrombin time was 11.4 s, partial thromoboplastin time 62.1 s, and fibrinogen 152 mg/dl. Fibrin degradation products were negative. Her urinalysis revealed a pH of 6.0, specific gravity of 1.007, 4+ protein, 3+ blood, and several granular casts. During hospitalization her creatinine rose to 4.5 mg/dl.

Samples

Two plasma and two urine samples were obtained from both patients with HIV-HUS during acute stages of the disease, while the patients were not undergoing dialysis treatment and the serum creatinine levels were approximately twice the normal values according to the patients'age. Control samples were obtained from HIV-infected children without renal disease (n=20) and children with HIV nephropathy (*n*=5). In addition, to determine whether the changes in bFGF levels were specific for HIV-HUS, these results were compared with the results obtained in a previous study of ten Argentinean children with Shiga toxin-induced HUS [17].

Renal sections

For immunohistochemistry and receptor binding studies, renal sections obtained at autopsy or renal biopsy from both patients with HIV-HUS were compared with renal sections from six HIV-infected children without renal disease; two children with complex congenital heart disease with secondary renal failure and congested kidneys**.**

Cell cultures

Human mesangial cells were isolated from renal glomeruli derived from patient no. 2 following procedures described previously [19]. Briefly, the kidney cortex was minced into 1-mm fragments and passed through a graded series of metal sieves. The resulting material, containing approximately >95% glomeruli by microscopic examination was incubated with collagenase (Sigma type IV, Sigma, St Louis, Mo., USA) at 37°C. After centrifugation pelleted glomeruli were resuspended in 50% Dulbecco's modified Eagles medium, 50% Ham's F12 (Biofluid, Rockville, Md., USA) containing 4.5 g/l glucose, 20% heat-inactivated fetal calf serum (Gibco), 2 mM glutamate, 100 U/ml penicillin,10 µg/ml streptomycin, and 8 µM insulin (Sigma). Cells with mesangial cell morphology grew from the attached glomeruli. These cells were trypsinized, characterized, and expanded by plating them on tissue culture dishes as described before [19]. RTEc were isolated from the urine of both patients with HIV-HUS as previously described [20, 21]. Briefly, the urine was centrifuged and the cell pellets were plated on collagen-coated dishes and grown in the following selected RTEc culture media: Click RPMI (Quality Biologcial, Gaithersburg, Md., USA) supplemented with 1% fetal bovine serum (lot no. A9234J, Gemini Bioproducts, Calabasas, Calif., USA), 5 ml/500 ml (100×penicillin/streptomycin), 1 mM HEPES, 2 mM glutamine, insulin (5 µg/ml), transferrin (5 µg/ml), and dexamethasone 5×10^{-8} M (Sigma). Cells with RTEc morphology were identified and cloned using cloning rings, while all other attached cells were removed with a cell scraper. RTEc were grown to confluency, trypsinized, and characterized using standard markers for epithelial cells as previously described [19, 22].

Characterization of bFGF activity

Plasma and urine bFGF levels were measured by enzyme-linked immunoasorbent assays (R and D Systems, Minneapolis Minn., USA) as directed by the manufacture's instructions. The detection limit of bFGF was 0.5 pg/ml. Urine values were expressed as picograms bFGF per gram of creatinine. To confirm the presence of bFGF in the urine of children with HIV-HUS, urine samples were concentrated (5×) using Amicon filters (30 kilodaltons molecular weight cut-off) and mixed with heparin-Sepharose (HS) beads. After centrifugation, HS beads were washed with increasing concentrations of sodium chloride (NaCl) (0.6 M–2 M). Eluted samples (5×) were loaded onto sodium dodecyl sulfate-polyacrylamide gels and the presence of bFGF confirmed by Western blot analysis [12]. Cell proliferation was measured by [^{3}H] thymidine incorporation [12, 19]. All cell culture experiments were performed in triplicate and repeated at least three times.

Immunohistochemistry

The distribution of renal bFGF was determined by indirect immunohistochemistry using an avidin-biotin-peroxidase kit (Vector Laboratories, Burlingame, Calif., USA) as previously described [12, 23, 24]. We used affinity purified IgG fractions (2.5 µg/ml) from a rabbit polyclonal antibody directed against a unique peptide sequence of bFGF (generously provided by Dr. Andrew Baird, PRIZM Pharmaceuticals, San Diego, Calif., USA). The specificity of this antibody in human sections has been demonstrated before [24]. Controls included replacing the primary antibody with equiv-

alent concentrations of nonimmune IgG and using a primary antibody that had been blocked with a 20-fold molar excess of recombinant bFGF. An additional monoclonal antibody against bovine bFGF (Upstate Biotechnology, Lake Placid, N.Y., USA) was used to confirm the results. In addition, the following antibodies were used: mAb 10E4 (gift from Seikagaku America, Ijamsville, Md., USA) to detect HSPG, α-actin smooth muscle cell antigen (Sigma), cytokeratin, and proliferating cell nuclear antigen (PCNA) (Vector). The distribution and staining intensity pattern in renal glomeruli, tubules, and extracellular matrix was evaluated in 5 microscopic fields (10×) in each sections, using a score ranging from 0 (no staining) to 4+ (maximum staining). Only renal sections stained with the same antibody and under identical experimental conditions were compared.

Receptor binding studies

Binding studies were performed on frozen human renal sections by ex vivo autoradiography as previously described [12]. Briefly, sections were preincubated for 15 min in binding buffer (DMEM, 20 mM HEPES, pH 7.4 and 0.15% gelatin) followed by an incubation in the same buffer containing 0.25 nM [^{125}I]-bFGF (Amersham, Arlington Heights Ill., USA) at 4°C for 2 h. Nonspecific binding was determined by incubating sections with 5 µM unlabelled bFGF plus 30 µg/ml heparin or in the presence of 300 µg/ml heparin alone [17, 25]. Heparin was used as previously described by Moscatelli [25], because addition of unlabelled bFGF alone did not completely displace labelled bFGF. The affinity of renal binding sites for bFGF was measured by incubating sections with 0.25 nM [^{125}I]-bFGF in the absence or presence of two different concentrations of heparin (30 and 300 µg/ml). These concentrations of heparin were selected because they can displace bFGF from its low- and high-affinity binding sites in normal kidney sections [12]. Alternatively, the binding of bFGF to its low-affinity receptors was estimated by counting the bFGF binding before and after washing the renal sections with 2 mM NaCl, 20 mM HEPES, pH 7.4 for 1 min, as described by Moscatelli [25]. In addition, to confirm the binding of bFGF to renal HSPG, representative sections from control and HIV-HUS children were preincubated at 37°C with 0.1 units/ml heparitinase (Seikagaku America) for 1 h [26], and binding studies were performed at the end of this incubation period. The slides were then washed and the dried labelled sections, together with a set of [^{125}I] standards (Amersham), were exposed to [^{3}H]-labelled Ultrafilm (Amersham). The protein concentration in the renal sections was measured using the method of Miller et al. [27] as we have previously described [28]. In each experiment, equal amounts of iodinated growth factor or heparin were added to all sections, incubated under the same conditions, and autoradiographs were exposed for equal lengths of time. Films were developed with D19 Kodak developer for 5 min at 4°C and differences in binding sites were determined by computerized microdensitometry in specific kidney areas of 0.22 mm^2 as previously described [12]. The autoradiography measurements were performed in areas of similar protein concentration and expressed in optical density units. Ten different glomeruli were counted by computerized microdensitometry in each renal section, and these results were pooled to determine the mean optical density values corresponding to the bFGF binding located within glomeruli in each renal section.

Statistical analysis

Results are expressed as the mean±SD of values obtained in triplicate from at least three different experiments. Differences between groups were compared by Student's *t*-test. *P* values less than 0.05 were considered significant. When more than two means were compared, significance was determined by one-way analysis of variance followed by multiple comparisons using the Student-Neuman-Keul's test. For statistical analysis, values above or below the limits of detection of the assay were given the highest or lowest values according to each assay.

Results

Detection of bFGF

By immunoassay, the highest bFGF plasma levels were found in children with HIV-HUS, followed by children with HIV-associated nephropathy (Fig. 1a). Moreover, a plasma sample obtained 1 month before patient no. 2 developed HIV-HUS showed approximately 3 times lower bFGF levels than during the acute stages of HUS (38 and 137 pg/ml bFGF, before and during HUS, respectively). Unfortunately, no samples were obtained before patient no. 1 developed HUS.

The changes in bFGF urine levels followed the same pattern as bFGF plasma levels. The two patients with HIV-HUS had the highest levels of urine bFGF (mean±SD, 180,000±16,600 pg/g; $P<0.001$ when compared with all other groups). Western blot analysis confirmed the presence of bFGF in these HIV-HUS samples (Fig. 1b). Children with HIV-associated nephropathy ($n=5$) also had high bFGF levels (mean±SD 67, 600±8,400 pg/g; $P<0.001$

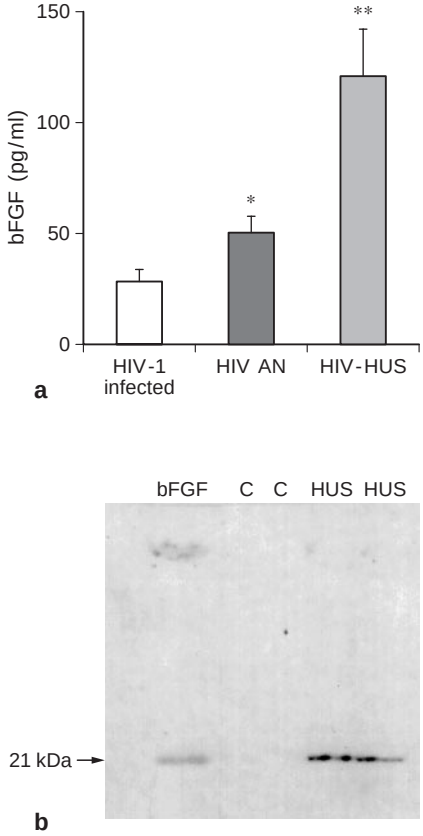

Fig. 1 **a** Mean±SD basic fibroblast growth factor (*bFGF*) plasma levels in 20 human immunodeficiency virus (*HIV*)-infected children, 5 children with HIV-associated nephropathy (*HIVAN*), and 2 children with HIV-hemolytic uremic syndrome (*HUS*). * $P<0.05$ compared with control children; ** $P<0.001$ compared with all other groups. **b** Western blot analysis for bFGF in urine samples derived from HIV-infected children (*C*) and children with HIV-HUS (HIV-HUS). Human recombinant bFGF (10 ng/ml) was used as a positive control. *kDa*, kilodaltons

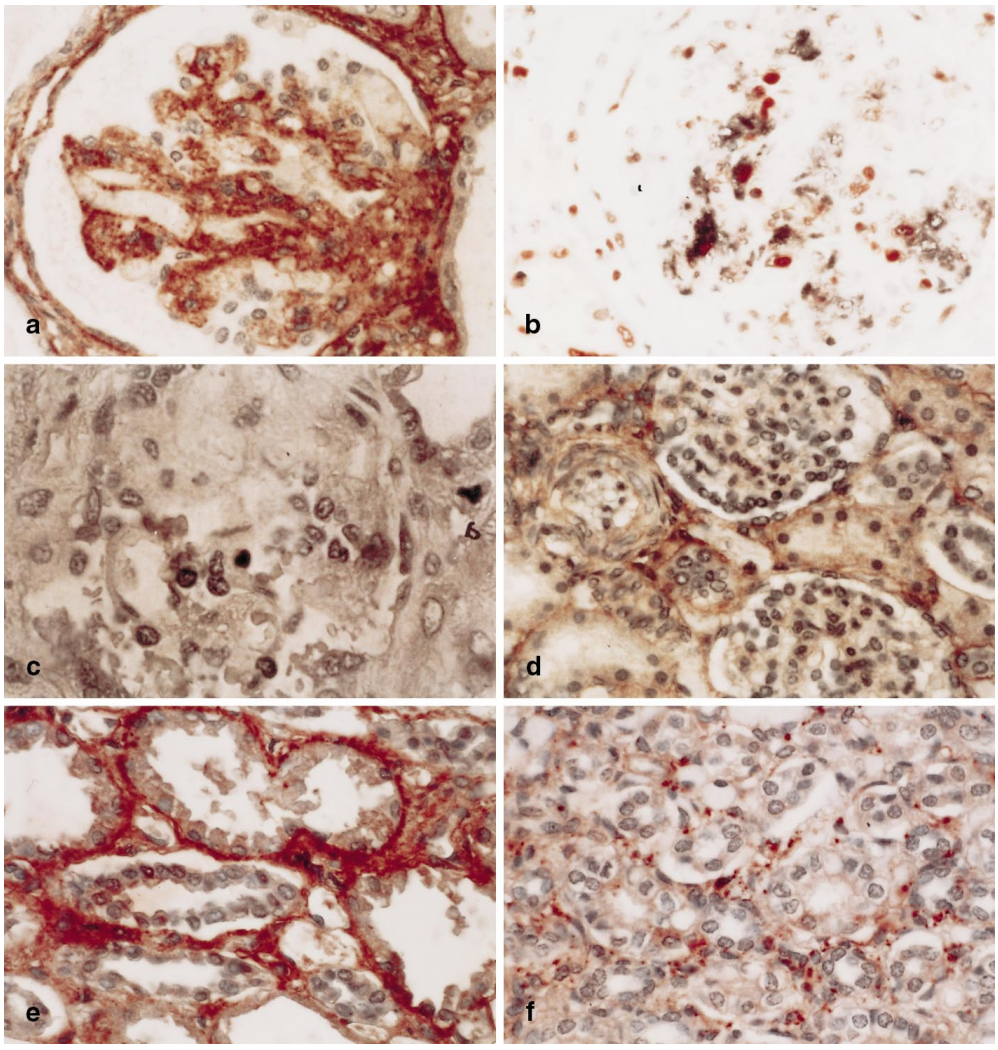

Fig. 2 **a** shows representative immunohistochemistry staining for bFGF in a renal glomerulus from one child with HIV-HUS (score 4);
b shows glomerular cells from the same child with HIV-HUS stained *red* with a proliferating cell nuclear antigen (*PCNA*) antibody and *blue* with an α-actin smooth muscle antibody;
c shows a glomerulus from the same child with HIV-HUS stained with the control antibody;
d shows representative staining for bFGF in a control renal section from a child with acute renal failure associated with a complex congenital heart disease; minimal bFGF staining is detected surrounding glomerular Bowman's capsule and peritubular renal extracellular matrix, but not in glomeruli;
e shows a representative HIV-HUS renal section showing staining for bFGF in the interstitium surrounding renal tubules;
f shows peritubular interstitial staining of bFGF in a control section.
(Magnification **a–c**, **e**, **f** ×500; **d**×360)

when compared with all other groups). However, HIV-infected children without renal disease (n=20) had mean (±SD) bFGF urine levels of 7,890±1,053 pg/g.

To determine whether the observed changes in bFGF plasma and urine concentrations were specific for HIV-HUS, we compared the bFGF levels measured in children with HIV-HUS with those measured in a previous study in ten Argentinean children with Shiga toxin-induced HUS [17]. We found that both clinical forms of HUS were associated with significant changes in bFGF plasma and urine concentrations of approximately similar magnitude (data not shown).

By immunohistochemistry, bFGF staining in HIV-HUS kidney sections was significantly increased in renal glomeruli (Fig. 2a, score 4) when compared with all control sections. Moreover, the accumulation of bFGF in renal glomeruli of children with HIV-HUS was associated with the presence of glomerular mesangial cells expressing the α-actin smooth muscle cell antigen and the PCNA antigen (Fig. 2b), suggesting the presence of an "activated" mesangial cell phenotype and cell proliferation. Renal glomeruli from HIV-HUS kidney sections stained with the control antibody did not show any specific staining (Fig. 2c). In contrast to sections from HIV-HUS, kidneys, bFGF staining was not detected in glomeruli from control samples (Fig. 2d, score O), while only minimal bFGF staining was detected surrounding proximal tubules (Fig. 2d, score 1). The staining of bFGF in the peritubular renal extracellular matrix, was also significantly increased in HIV-HUS renal sections (Fig. 2e, score 4), compared with all other control sections (Fig. 2f, score 1). Finally, renal vessels from children with HIV-HUS and control sections showed similar bFGF staining patterns (data not shown, average score 4 in both groups).

In addition, bFGF induced a fourfold increase in thymidine incorporation in cultured mesangial cells derived from patient no. 2 (control, 1,200±134 cpm/well vs. FGF, 4,156±242 cpm/well; P<0.001). These results demonstrate that mesangial cells derived from patients with HIV-HUS, and therefore exposed to high levels of bFGF and other growth factors known to modulate bFGF activity in vivo, also responded to bFGF mitogenic effects in vitro.

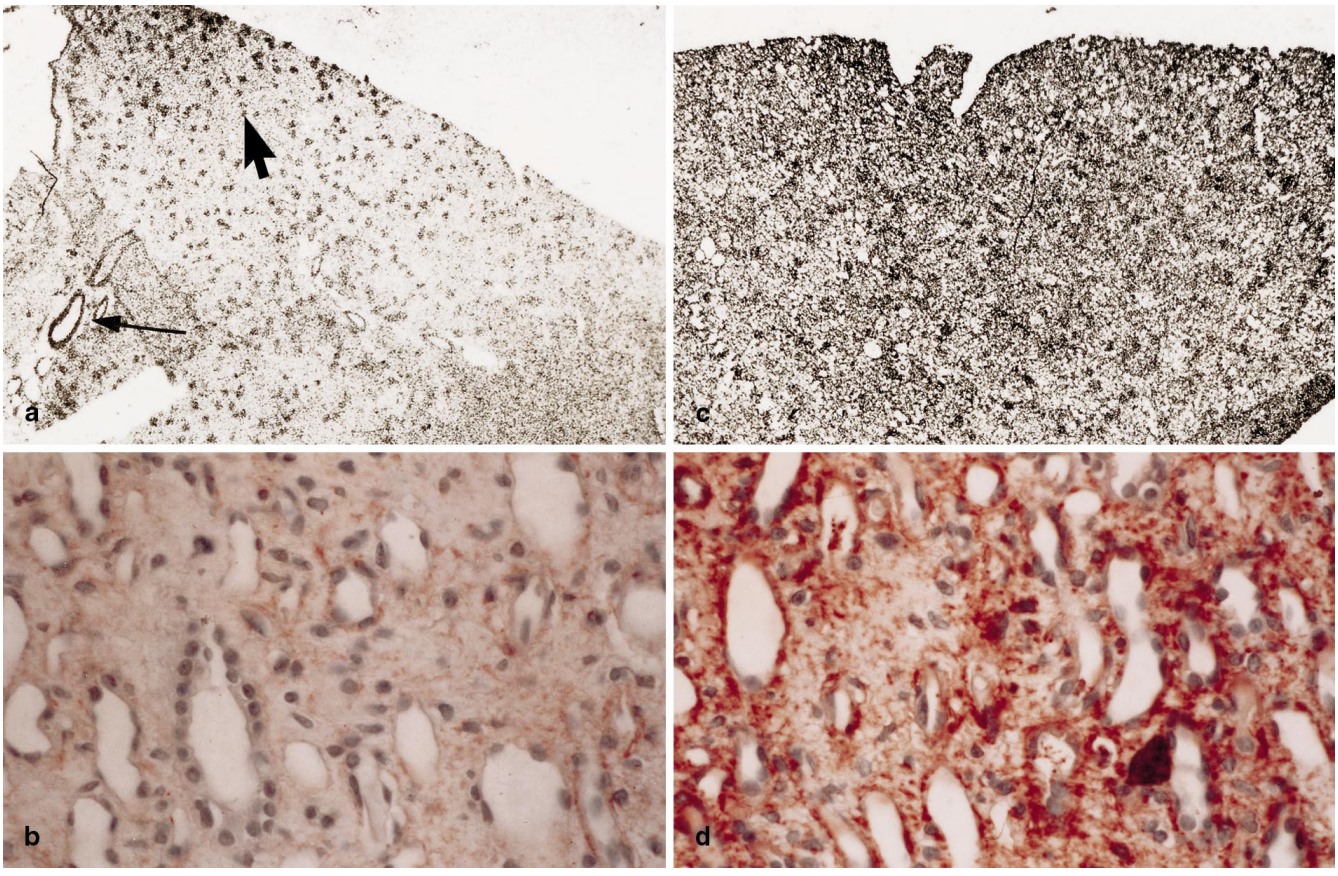

Fig. 3 In vitro autoradiography and immunohistochemistry studies in control and HIV-HUS kidney sections; **a** shows an autoradiographic study in a representative renal section from a control child with acute renal failure associated with a complex congenital heart disease; total bFGF binding was localized to glomeruli (*thick arrow*) and renal vessels (*thin arrow*); in contrast a representative autoradiography of a renal section from a child with HIV-HUS (**c**, patient 2) revealed total bFGF binding in similar areas, but significantly increased in the renal interstitium; **d** shows immunohistochemistry staining for heparan sulfate proteoglycans (HSPG) using the 10E4 antibody (gift from Seikagaku) in a renal section from a child with HIV-HUS (patient 1); **b** shows elimination of the HSPG staining by heparitinase treatment (0.1 unit/ml, Seikagaku) in a renal section from the same child. (Magnification **a** and **c**, ×50; **b** and **d**, ×360)

Effects of bFGF on RTEc

Since bFGF was localized in the renal interstitium close to RTEc, we studied whether bFGF could stimulate the growth of RTEc isolated from both HIV-HUS patients. Primary RTEc derived from the urine of both HIV-HUS children expressed bFGF, proliferated rapidly, and formed tubular-like structures when cultured *on* type I collagen gels (data not shown). These findings suggest that these RTEc can penetrate the collagen gels. In a previous study, we have shown that primary RTEc derived from HIV-transgenic mice with renal disease formed tubular-like structures when cultured *on* type I collagen gels, while primary RTEc derived from littermate control mice only formed similar tubular-like structures when exposed to exogenous bFGF [17]. In addition, high levels of bFGF were found in the culture media of the human primary RTEc isolated from HIV-HUS patients (average 20 pg/ml 1×10^6 cells). Finally, human recombinant bFGF induced proliferation of RTEc derived from both HIV-HUS affected children by approximately twofold (control RTEc, $75\pm11\times10^3$ cell/well vs. bFGF-treated RTEc, 156 ± 24 10^3 cell/well; $P<0.001$). Since under normal conditions, epithelial cells can not be cultured from the urine of children, additional studies will be required to define whether similar changes can be seen in urinary RTEc isolated from other renal acute pediatric renal diseases.

Basic FGF low-affinity receptors

By ex vivo autoradiography studies performed in control kidney sections, the highest affinity binding sites were localized in glomeruli and vascular structures in the renal cortex (Fig. 3a). Renal sections from children with HIV-HUS showed an average 39% increase in glomerular bFGF binding sites compared with control sections (HIV-HUS, 144±12 units; control 86±7 OD units, $*P<0.05$). Nonspecific binding was less than 10% of the total binding. In HIV-HUS kidneys, bFGF binding was detected in glomeruli and renal vessels, but was particularly increased in the renal interstitium (controls, 8±3 vs. HIV-HUS 32±6 OD units; $P<0.001$; Fig. 3a and c). The binding of bFGF to these sites could not be completely dis-

placed by even a 100-fold excess of unlabelled bFGF, suggesting the presence of a very large reservoir of bFGF low-affinity binding sites that could not be saturated by bFGF. This binding however, was partially displaced by incubating the sections with 30 µg/ml heparin and completely displaced by a heparin concentration of 300 µg/ml or by heparitinase treatment (0.1 U/ml) (data not shown). These experiments clearly demonstrate that bFGF binds to high-affinity receptors and to renal HSPG. Immunohistochemisty studies performed on kidney sections using a monoclonal antibody that specifically recognizes HSPG (mAb10E4) (Fig. 3b and d) confirmed the results obtained by autoradiography. The staining of HSPG in renal sections from children with HIV-HUS (Fig. 3d) was eliminated by heparitinase treatment (Fig. 3b).

Discussion

bFGF is an 18-kilodalton protein belonging to the family of heparin-binding FGFs [13–15]. Unlike most other members of the FGF family, bFGF lacks a conventional signal sequence for secretion and is primarily a cell-associated protein. Thus, its presence in the circulation suggests an undergoing process of tumor growth, angiogenesis, or vascular injury [15, 18, 29]. Here, we demonstrate the presence of very high circulating and renal tissue levels of biologically active bFGF in two children with HIV-HUS. These findings, when viewed in the context of previous published studies of HIV-1 transgenic mice [12] and children with classic HUS [17], suggest that bFGF and its low-affinity receptors may have a relevant clinical role during the acute stages of HIV-HUS.

Recent studies have demonstrated an important role of bFGF in the pathogenesis and progression of renal disease. In a rat model of immunologically mediated glomerulonephritis, bFGF released by injured cells induced proliferation of mesangial cells [30]. Long-term treatment of normal rats with bFGF induces podocyte injury and focal segmental glomerulosclerosis [31, 32], two lesions frequently seen in HIV-associated nephropathy. Moreover, bFGF has been linked to the development of tubulointerstitial injury in humans [26] and HIV-1 transgenic mice [12]. These studies suggest that bFGF is not only a relevant growth factor for endothelial cells, but an important modulator of renal mesangial and tubular epithelial cell growth and regeneration.

The accumulation of bFGF in glomeruli and renal extracellular matrix in HIV-HUS affected children contrast with the distribution of renal bFGF in the other control children. Under normal conditions, bFGF is detected in glomerular Bowman's capsule and the wall of blood vessels, with minimal staining surrounding proximal tubules or renal extracellular matrix. In previous studies, a similar distribution of bFGF was found in normal [33] or diseased [26] adult human kidneys, with the exception of patients with renal cell carcinoma [33]. However, in HIV-HUS kidneys, bFGF was significantly increased in glomeruli and the renal tubulointerstitium. In a previous study [17] we found similar high circulating and renal tissue levels of bFGF in children undergoing the acute stages of classic or Shiga toxin-induced HUS. Thus, these preliminary results support the hypothesis that bFGF released by injured glomerular endothelial cells accumulates in the circulation and kidneys of children with HUS leading to the proliferation of renal cells. Moreover, since the mitogenic effects of bFGF may vary depending on the number of bFGF low-affinity receptors expressed in the target tissues [12–14] and the presence of preexisting injury in these tissues [34], HIV-infected children undergoing the acute stages of HUS may be more sensitive to bFGF mitogenic effects than HIV-negative children with classic HUS or other acute pediatric renal diseases. However, only a prospective clinical study performed in a large population of children with HIV-HUS, classic HUS, and other acute pediatric renal diseases will define the role of bFGF in each of these circumstances.

At least four mechanisms may explain the release and accumulation of bFGF in HIV-infected children. First, bFGF is abundantly expressed in human fetal kidneys [24], and young children express higher renal levels of bFGF than adults. Second, vascular injury is an important component of the pathogenesis of the acquired immunodeficiency syndrome [8–10], even in the absence of HUS. Thus, bFGF may be constantly released during this process, leading to higher bFGF plasma levels in HIV-infected children. Third, HIV-transgenic mice with renal disease express an increased number of renal bFGF low-affinity binding sites [12]. These bindings sites are HSPG localized on the cell surface, extracellular matrix, and basement membranes [12, 14, 23, 24, 33], and may act as a "sink" trapping circulating bFGF [12]. When bFGF is bound to HSPG, it becomes an integral part of the cell or extracellular matrix structure, and it is protected from proteolytic degradation and inactivation [13, 14, 25]. However, HIV-infected mononuclear cells infiltrating the kidney produce and release proteases capable of releasing bFGF from the extracellular matrix [35, 36]. Thus, the presence of chronic and persistent HIV-induced endothelial injury and recruitment of mononuclear cells, in combination with an increased number of bFGF low-affinity binding sites, may explain the high plasma levels of bFGF found in children with HIV-associated nephropathy. All these mechanisms are typical features of HIV-HUS but may be clinically relevant in other forms of HUS as well. Finally, it could be argued that the changes in renal function per se may lead to the accumulation of bFGF in the circulation and the kidney. However, since bFGF is normally not released into the circulation by normal endothelial cells, it is unlikely that changes in renal function per se, in the absence of endothelial injury, could explain the presence of such high levels of bFGF in children with HIV-HUS. This notion is supported by the low levels of bFGF detected by immunohistochemistry in our two control kidney sections from children with hemodynamically induced acute renal failure.

Several studies support the concept that bFGF may have multiple roles in the pathogenesis of HUS. For ex-

ample, bFGF is known to modulate endothelial cell growth, the activity of several proteases, and the plasminogen-plasmin pathway involved in vascular regeneration [37]. High levels of plasminogen activator inhibitor-1 (PAI-1) are found in the circulation [38] and renal glomeruli [39] of HUS-affected children, where PAI-1 favors the accumulation of fibrin in glomerular capillaries. Thus, the increased levels of bFGF may influence glomerular fibrinolytic mechanisms known to play a crucial role in tissue remodelling and vascular regeneration. In addition, a recent study has shown that plasma samples derived from adult patients with HIV-thrombotic thrombocytopenic purpura or sporadic forms of HUS induce apoptosis in renal microvascular endothelial cells [40]. bFGF inhibits endothelial cell apoptosis by Bcl-2-dependent and -independent mechanisms [41, 42], but it may induce apoptosis in other cell types [43, 44]. Based on these studies, it is tempting to speculate that high levels of bFGF may play an important role in modulating soluble and local tissue factors involved in apoptosis in renal cells. Here, we have shown that RTEc and mesangial cells isolated from children with HIV-HUS, and therefore exposed to high levels of bFGF in vivo, continued to respond to bFGF mitogenic effects in vitro. Thus, these findings strongly suggest that bFGF is an important renal mitogen during the acute stages of HIV-HUS, and support the notion that the urine levels of bFGF may be a useful marker to monitor the proliferative response of the kidney during the nonoliguric acute stages of HIV-HUS [7]. However, at present, we can not determine the exact clinical role that bFGF might have in the pathogenesis of HUS. We speculate that during the initial stages of HUS, bFGF may have the protective role of facilitating the regeneration of endothelial, mesangial, and epithelial cells, and of preventing apoptosis in these cells. Subsequently, if too much bFGF remains accumulated in renal glomeruli or peritubular extracellular matrix, bFGF may facilitate the development of focal segmental glomerulosclerosis [31, 32] and chronic renal tubulointerstitial lesions [12, 26].

In summary, we have shown high levels of bFGF in the circulation and kidneys from children with HIV-HUS. Although a prospective clinical study in a large number of patients is necessary to confirm these results, the accumulation of bFGF in HIV-transgenic mice with renal disease [12] and in children with Shiga toxin-induced HUS [17], suggests that these findings may be clinically relevant. Moreover, although these changes appear not to be restricted to HIV-HUS, the HIV-associated cytokine milieu may induce specific changes in the expression of renal bFGF low-affinity receptors and affect the clearance of bFGF, leading to long-term renal lesions. Thus, based on these data, we hypothesize that HIV-infected children may be more sensitive to the renal mitogenic and fibrogenic effects of bFGF. This hypothesis may explain at least in part the marked renal proliferative response in HIV-HUS kidneys, and suggests that bFGF could be a good marker to monitor the outcome of HIV-HUS in children. Hopefully, a more-clear understanding of the mechanisms responsible for bFGF binding, cleareance, and pathogenic effects may facilitate the development of novel therapies to prevent the progression of renal disease and the high mortality rates associated with HIV-HUS in children.

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