

## Brief Report

## NEPHROPATHY AND ESTABLISHMENT OF A RENAL RESERVOIR OF HIV TYPE 1 DURING PRIMARY INFECTION

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**H**UMAN immunodeficiency virus type 1 (HIV-1)-associated nephropathy is the chief cause of chronic renal disease in patients with HIV-1 infection and is now the third leading cause of end-stage renal disease in blacks 20 to 64 years of age.<sup>1,2</sup> These patients typically have proteinuria followed by a reduction in the glomerular filtration rate that progresses to end-stage renal disease in a few weeks or months. HIV-1-associated nephropathy is characterized morphologically by focal segmental glomerulosclerosis, tubular microcysts, interstitial fibrosis, and inflammation.<sup>2-5</sup>

The pathogenesis of HIV-1-associated nephropathy is poorly understood, but increasing evidence suggests it is due to HIV-1 infection of renal tissue. Transgenic mice expressing a deletion construct of the HIV-1 provirus have morphologic changes in the kidney that are identical to the disease in humans.<sup>6</sup> Reciprocal transplantation studies demonstrate that HIV-1-associated nephropathy develops only in kidneys expressing the transgene.<sup>7</sup> Recently, HIV-1 has been detected in glomerular podocytes and renal tubular epithelial cells in patients with HIV-1-associated nephropathy.<sup>8</sup> In this report, we provide evidence that the kidney may be an important long-term reservoir for the virus.

### CASE REPORT

A 35-year-old man was hospitalized because of a six-week history of fatigue, weight loss, abdominal pain, diarrhea, and night sweats. He had received multiple antibiotics with no benefit. Several days before admission, a rash and cervical and inguinal lymphadenopathy developed. He had had two negative tests for HIV-1 infection within the previous 12 months, most recently 4 months before admission.

Physical examination revealed a well-developed, well-nourished man. His temperature was 38°C, and he was normotensive. Large,

firm, nontender lymph nodes were palpable in the cervical, submandibular, and inguinal regions. His rash consisted of discrete erythematous papules on the trunk, arms, and legs. Laboratory studies revealed a white-cell count of 13,000 per cubic millimeter (34 percent neutrophils, 50 percent lymphocytes, and 8 percent monocytes), a hemoglobin concentration of 13 g per deciliter, a platelet count of 263,000 per cubic millimeter, a serum creatinine concentration of 1.9 mg per deciliter (168 μmol per liter), a serum albumin concentration of 1.6 g per deciliter, and a serum γ-glutamyltransferase concentration of 181 U per liter (normal range, 10 to 54). Serum bilirubin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase concentrations were normal. Urine dipstick testing revealed a protein concentration of more than 300 mg per deciliter, and microscopical analysis showed 5 to 10 red cells per high-power field, but there were no casts or eosinophils. A chest x-ray film and an electrocardiogram showed no abnormalities.

An enzyme-linked immunosorbent assay and a Western blot assay for HIV-1 antibodies were positive, the plasma HIV-1 RNA level was more than 750,000 copies per milliliter, and the CD4 cell count was 459 per cubic millimeter. The findings on a skin biopsy were consistent with the presence of a viral exanthem. Blood cultures were negative, as were serologic tests for systemic lupus erythematosus and active infection with hepatitis B or C virus. Urinary protein excretion was 17 g per day. The patient's serum creatinine concentration rose to 3.8 mg per deciliter (336 μmol per liter) after admission. A kidney biopsy was performed. The patient's fever persisted, the rash became more prominent, and the serum creatinine concentration continued to rise. Treatment with zidovudine, lamivudine, and nelfinavir was begun on the 14th hospital day. By the 15th hospital day, the patient's serum creatinine concentration had risen to 6.3 mg per deciliter (557 μmol per liter) and his rash had become pruritic and morbilliform in appearance. Hemodialysis was initiated on the 18th hospital day, after which his renal function stabilized and later improved. After hospitalization for 39 days, the patient was discharged while taking stavudine, lamivudine, and nelfinavir. His serum creatinine concentration had fallen to 2.8 mg per deciliter (248 μmol per liter), and his serum albumin concentration was 1.9 g per deciliter.

After discharge the patient's plasma HIV-1 RNA level became undetectable (<50 copies per milliliter), his glomerular filtration rate increased, and his proteinuria decreased. After six weeks of highly active antiretroviral therapy, the serum creatinine concentration was 1.4 mg per deciliter (124 μmol per liter) and urinary protein excretion was 1.5 g per day. A second kidney biopsy was performed three months after the initiation of antiretroviral therapy.

### METHODS

The two biopsy specimens were prepared for light microscopy, immunofluorescence microscopy, and electron microscopy according to standard techniques and stained with hematoxylin and eosin, periodic acid-Schiff, and trichrome. A pathologist who did not know the patient's history or that the two biopsy specimens were from the same patient evaluated the specimens.

### In Situ Hybridization

Tissue fixation, riboprobe preparation, and in situ hybridization for HIV-1 messenger RNA (mRNA) were performed as previously described.<sup>7,8</sup> Tissue was examined with the use of antisense and sense probes derived from the *gag* region. To generate the probe, a 359-bp fragment obtained by the polymerase chain reaction (PCR) from the HXB<sub>2</sub> isolate (nucleotides 1031 to 1390) was subcloned into a pGEM-T Easy vector (Promega, Madison, Wis.). Renal-biopsy tissue from a patient with systemic lupus erythematosus was processed in an identical fashion as a negative control.

### Immunohistochemical Analysis

Immunostaining for synaptopodin and Ki-67, an antigen expressed only by proliferating cells, was performed on formalin-fixed, paraffin-embedded tissue as previously described.<sup>9,10</sup>

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### PCR for Circular Forms of Viral DNA

Circular forms of viral DNA were detected with the use of a previously described PCR technique and primers.<sup>8,11</sup> This approach selectively amplifies circular forms of viral DNA that contain two copies of the long-terminal-repeat segments. DNA extracted from the biopsy specimens was added to an amplification mixture that included AmpliTaq buffer, 250 ng each of sense and antisense primers, 200  $\mu$ M deoxynucleoside triphosphate, and 1  $\mu$ l of AmpliTaq DNA polymerase. Aliquots of the PCR products were resolved on agarose gels and Southern blotted on nylon membranes. The blots were hybridized with use of a probe labeled with phosphate-32 that was internal to the PCR primers corresponding to the long-terminal-repeat sequence of the HXB<sub>2</sub> isolate.

### RESULTS

In the biopsy specimen obtained before the initiation of highly active antiretroviral therapy, two thirds of the 38 glomeruli examined had areas of capillary collapse and focal glomerulosclerosis (Fig. 1). There was also diffuse tubulointerstitial edema, numerous dilated tubules that contained large proteinaceous casts, forming microcysts, and moderate interstitial infiltration by lymphocytes and plasma cells with patchy interstitial fibrosis (Fig. 1A and 1C). Electron microscopy revealed prominent hypertrophy of podocytes with extensive effacement of foot processes and numerous endothelial tubuloreticular structures (not shown). The combination of findings was consistent with the presence of severe HIV-1-associated nephropathy.

The biopsy specimen obtained during antiretroviral therapy contained nine glomeruli. Two of the nine glomeruli contained lesions of segmental sclerosis and discrete scars, but none had features of acute collapse or hyperplasia of podocytes (Fig. 1B and 1D). There was a marked decrease in the severity of the tubulointerstitial disease, with no identifiable tubule microcysts, almost complete disappearance of the interstitial inflammatory infiltrate (Fig. 1B), and mild-

to-moderate patchy fibrosis (Fig. 1B and 1D). Electron microscopy revealed nearly complete restoration of podocyte foot processes, with the disappearance of endothelial tubuloreticular inclusions (not shown). The combined findings on light, immunofluorescence, and electron microscopy were consistent with the presence of mild, relatively inactive HIV-1-associated nephropathy.

Kidney-biopsy specimens obtained before and during antiretroviral therapy were stained for synaptopodin, a marker of mature podocytes, and the proliferation marker Ki-67. Before therapy, Ki-67 was detected focally in podocytes and tubular epithelial cells, particularly in areas of microcystic dilatation (Fig. 1E). During therapy, Ki-67 immunoreactivity disappeared as tubular architecture was restored (Fig. 1F). In the pretreatment biopsy specimen, staining for synaptopodin was weak or absent in the podocytes of both collapsed and noncollapsed glomeruli (Fig. 1G), an abnormal pattern that has been described previously in kidney-biopsy specimens from patients with HIV-1-associated nephropathy.<sup>9</sup> During antiretroviral therapy, the normal expression of synaptopodin along the base of the podocytes was restored in all glomeruli (Fig. 1H).

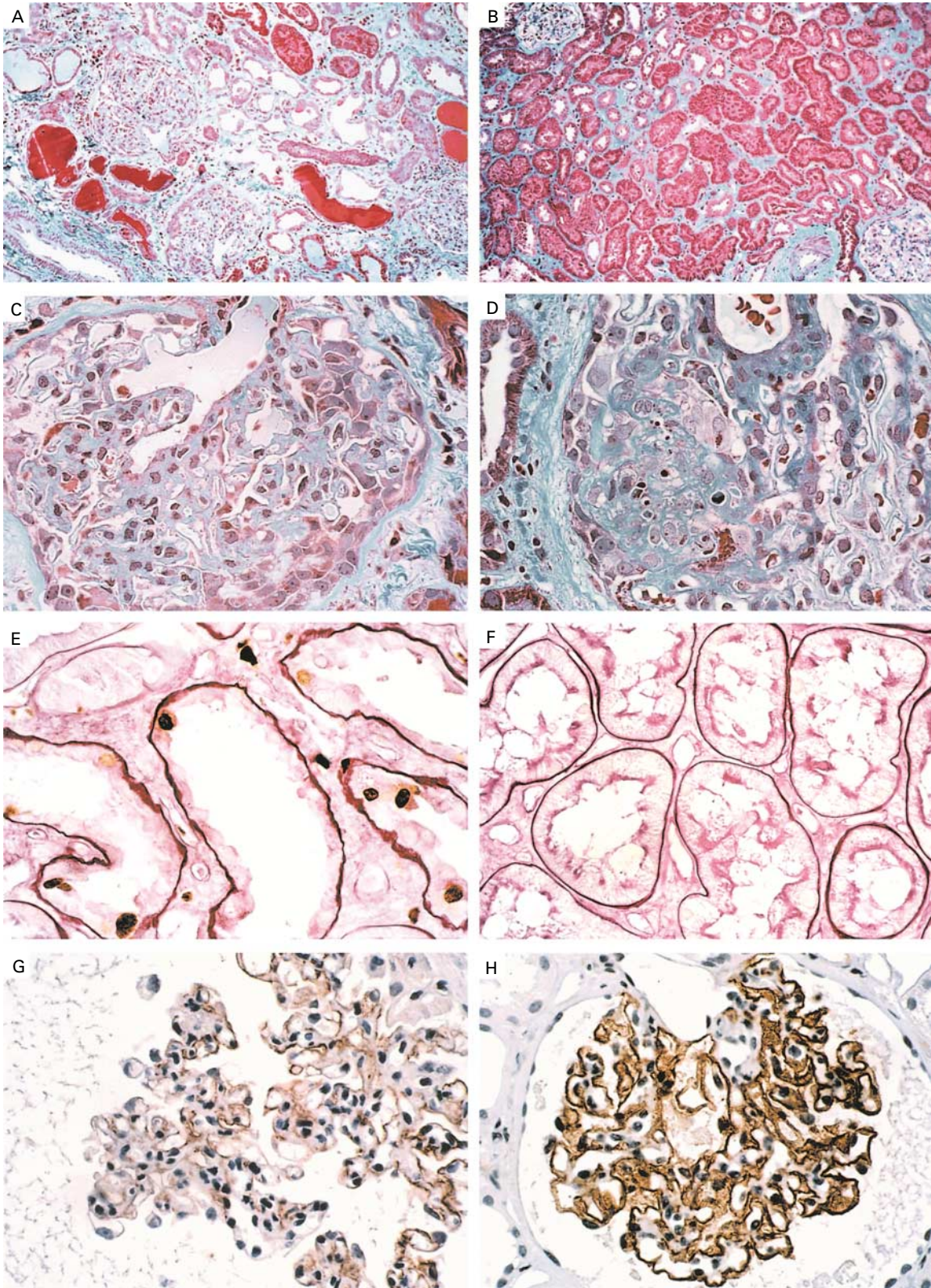
In situ hybridization for HIV-1 mRNA before and during antiretroviral therapy (Fig. 2A and 2B, respectively) revealed HIV-1 RNA in tubular epithelial cells and glomerular podocytes. The mRNA was detected with use of a probe derived from the *gag* region, indicating the presence of full-length viral mRNA. The number of renal epithelial cells that expressed viral mRNA was similar before and during treatment (Fig. 2A and 2B, respectively).

DNA was extracted from both renal-biopsy specimens (Fig. 3). The specimen obtained before treatment revealed a circular, unintegrated form of viral

**Figure 1 (facing page).** Kidney-Biopsy Specimens Obtained before and after the Initiation of Highly Active Antiretroviral Therapy. Panels A and C are low-power and high-power views, respectively, of the pretreatment biopsy specimen. Panel A shows one of three glomeruli with collapsing sclerosis and marked hyperplasia of podocytes (trichrome stain,  $\times 125$ ). The tubules are separated by edema, mild fibrosis, and patchy interstitial inflammatory infiltrates. Many proximal tubules show degenerative changes, and there are focal tubular microcysts containing large casts. Panel C shows a glomerulus with segmental collapse of the glomerular tuft and hyperplasia of the overlying podocytes (trichrome stain,  $\times 400$ ). Panels B and D are low-power and high-power views, respectively, of the biopsy specimen obtained three months after the initiation of highly active antiretroviral therapy. Panel B shows normal glomeruli and mild focal interstitial fibrosis, with restoration of normal tubular architecture (trichrome stain,  $\times 125$ ). No tubular microcysts or interstitial inflammation is apparent. In Panel D, a glomerulus contains a discrete segmental scar (trichrome stain,  $\times 400$ ). Some of the overlying podocytes contain protein-resorption droplets, but without the hyperplasia that was prominent before the initiation of treatment. Panels E, F, G, and H show the results of immunohistochemical staining of kidney-biopsy specimens obtained before and after the initiation of antiretroviral therapy. In the pretreatment biopsy specimen (Panel E), many nuclei in the renal tubular epithelial cells stain for Ki-67. There is diffuse loss from the proximal tubules of brush border staining for periodic acid-Schiff (periodic acid-Schiff counterstain,  $\times 400$ ). In the biopsy specimen obtained after the initiation of antiretroviral therapy (Panel F), a representative field shows no staining for Ki-67. The proximal tubular brush border has been restored (periodic acid-Schiff counterstain,  $\times 400$ ). Immunostaining for synaptopodin in the pretreatment biopsy specimen shows weak staining or no staining in the podocytes of a collapsed glomerulus (Panel G,  $\times 400$ ). There is strong, global positivity for synaptopodin in the podocytes of a representative glomerulus from the biopsy specimen obtained after three months of highly active antiretroviral therapy (Panel H,  $\times 400$ ).

Before Treatment

After Treatment



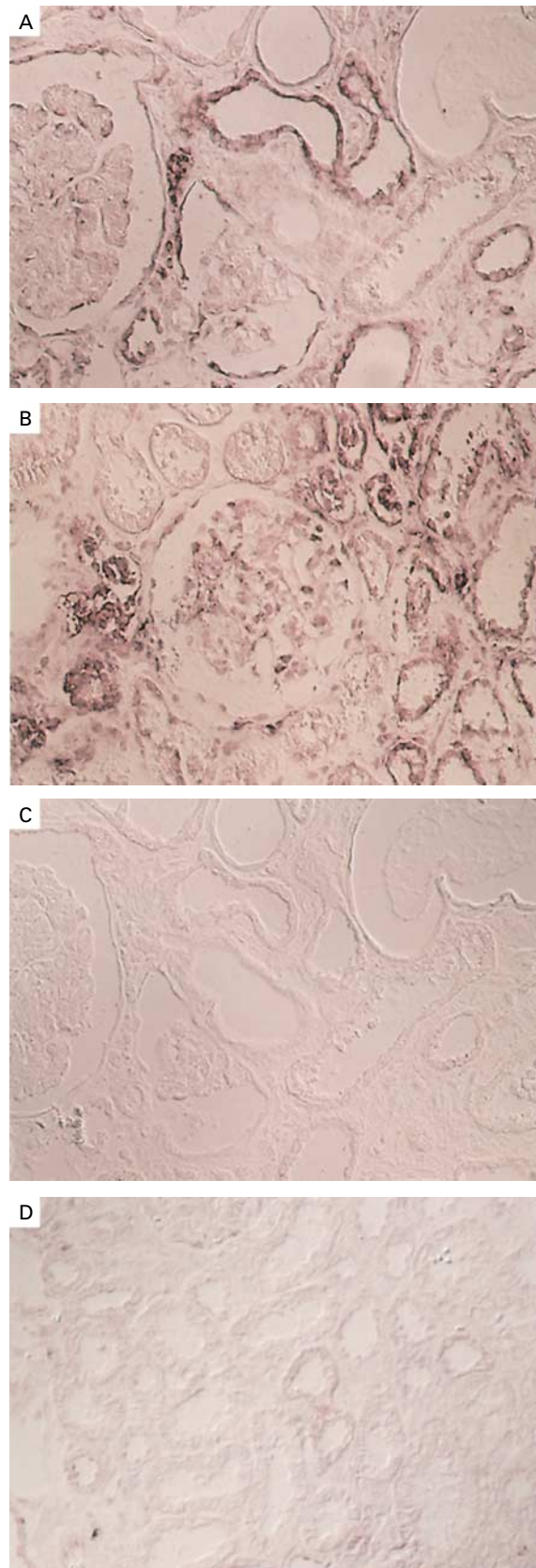
DNA containing two long terminal repeats, as indicated by the expected 120-bp PCR product. This circular form of viral DNA is a head-to-tail ligation of the long terminal repeats that occurs after reverse transcription and nuclear import of proviral DNA. Because these extrachromosomal forms of DNA have a short half-life in replicating cells, they are considered to indicate the recent infection of a cell. There were no detectable circular forms of viral DNA that contained two long terminal repeats in the specimen obtained during antiretroviral treatment (Fig. 3), suggesting that antiretroviral therapy blocked the infection of cells in the kidney.

### DISCUSSION

In our patient, HIV-1–associated nephropathy occurred early in the course of infection, in contrast to the pattern in most previously reported cases. Furthermore, the infection in this patient responded well to highly active antiretroviral therapy. Surprisingly, although there was fibrosis, many of the changes, particularly the collapsing glomerular disease and microcyst formation, were reversible. Finally, the kidney appears to be an important reservoir for HIV-1 infection, because the intracellular expression of viral RNA persisted despite antiretroviral therapy.

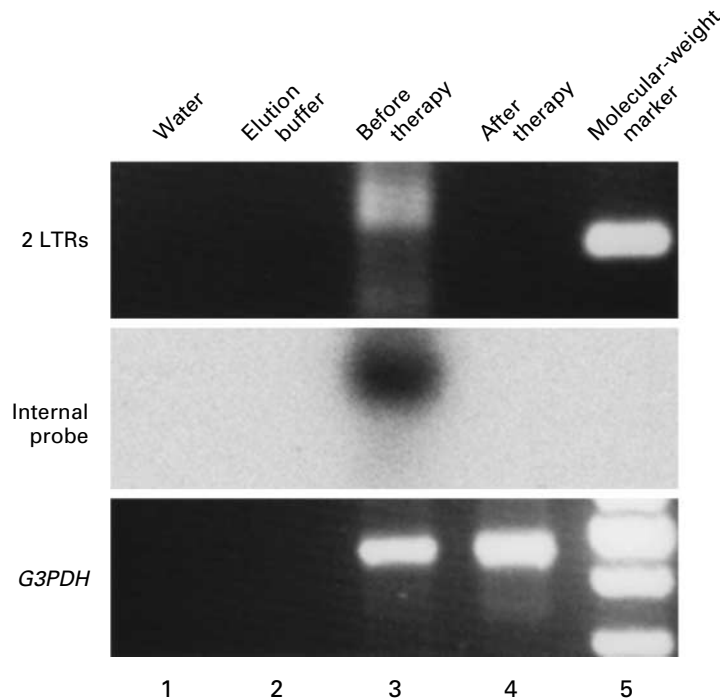
We previously reported that HIV-1–associated nephropathy occurs relatively late in the course of infection, after the development of an acquired immunodeficiency syndrome–defining condition.<sup>12</sup> This finding was based on our studies and on those reported by others in which all patients with HIV-1–associated nephropathy had either an opportunistic infection or a CD4 count of less than 200 per cubic millimeter when HIV-1–associated nephropathy was diagnosed.

Observational studies have suggested that zidovudine may be of benefit in patients with HIV-1–associated nephropathy, but kidney biopsies were not performed to confirm the diagnosis of HIV-1–associated nephropathy.<sup>13,14</sup> Wali et al. reported substantial improvement in kidney function in a patient with HIV-1–associated nephropathy who was treated with high-



**Figure 2.** HIV-1 RNA in Situ Hybridization of Kidney-Biopsy Specimens Obtained before and after the Initiation of Highly Active Antiretroviral Therapy ( $\times 50$ ).

The use of an antisense probe generated from the *gag* region demonstrates the presence of HIV-1 RNA in tubular cells and podocytes as well as in interstitial inflammatory cells before (Panel A) and after (Panel B) the initiation of highly active antiretroviral therapy. A control sense probe showed no staining of the pretreatment biopsy specimen (Panel C), and hybridization of renal tissue from an HIV-1–negative patient with systemic lupus erythematosus with the antisense probe showed no staining (Panel D).



**Figure 3.** Amplification of Circular Forms of HIV-1 DNA Containing Two Long Terminal Repeats in Kidney-Biopsy Specimens Obtained before and after the Initiation of Highly Active Antiretroviral Therapy.

Circular forms of DNA containing two long terminal repeats (2 LTRs) are present in DNA extracted from the biopsy specimen obtained before treatment (lane 3), as indicated by the 120-bp signal on the ethidium bromide–stained gel (top panel) that was detected on Southern blot hybridization with a  $^{32}\text{P}$ –end-labeled internal probe (middle panel), but not in DNA extracted from the biopsy specimen after the initiation of antiretroviral therapy (lane 4) or in the water and elution-buffer controls (lanes 1 and 2, respectively). Amplification of the cellular gene glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) showed that the amounts of DNA were similar in the pretreatment and post-treatment samples (bottom panel).

ly active antiretroviral therapy.<sup>15</sup> In both that patient and our patient, the kidney disease was diagnosed almost immediately after its onset, a factor that may have contributed to the dramatic clinical response.

In our patient, the post-treatment biopsy indicated almost complete resolution of the hypertrophy of podocytes and glomerular collapse and normalization of tubular architecture. In addition, treatment reversed the changes in epithelial proliferation and differentiation markers. Barisoni et al. demonstrated that in patients with collapsing glomerulosclerosis, especially those with HIV-1–associated nephropathy, mature podocytes dedifferentiate.<sup>9</sup> Synaptopodin, normally found only in mature podocytes, is lost in HIV-1–associated nephropathy, and the podocytes undergo proliferation, as indicated histochemically by the expression of Ki-67. We have demonstrated that the loss of synaptopodin and the proliferation that en-

sues are reversible events. We also observed a reduction in the degree of interstitial infiltration during highly active antiretroviral therapy, as was also noted by Wali et al.<sup>15</sup> Therefore, interstitial inflammation may contribute to the nephropathy and may respond to therapy. As we recently reported, HIV-1 RNA in situ hybridization showed that the virus was present in both tubular and glomerular epithelial cells.<sup>8</sup> The persistence of viral transcription during therapy at a time when the symptoms of the disease were improving suggests that the small amounts of viral proteins generated by these transcripts are insufficient to sustain the nephropathy. The observations in our patient suggest that a reduction in plasma HIV-1 RNA levels and viral infection in the kidney, as indicated by the absence of circular forms of DNA in the kidney, can ameliorate the morphologic and functional abnormalities of HIV-1–associated nephropathy.

This response to early intervention is evidence of the merits of actively screening HIV-1–infected patients for proteinuria.

Our finding of viral transcripts in renal epithelial cells even in the setting of effective therapy is similar to a report of the persistence of spliced and unspliced HIV-1 mRNA in peripheral-blood mononuclear cells in such patients.<sup>16</sup> Our results suggest that renal epithelial cells, like these other cells, may be a persistent reservoir of HIV-1 RNA transcription and that any interruption in therapy could lead to the rapid formation of infectious virions.<sup>17</sup>

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