Recent Progress in HIV-Associated Nephropathy

MICHAEL J. ROSS and PAUL E. KLOTMAN
Division of Nephrology, The Mount Sinai School of Medicine, New York, New York.

The association between HIV and renal disease was first reported in 1984 by investigators in New York City and Miami, who reported a series of HIV-1-seropositive patients who developed a renal syndrome characterized by progressive renal failure and proteinuria (1–3). The most common kidney biopsy finding was focal segmental glomerulosclerosis (FSGS). During the next several years, the existence of a specific HIV-associated nephropathy (HIVAN) was debated, in part, because of its similarity to heroin nephropathy and the frequent occurrence of intravenous drug use in this population (4). Finding HIVAN in patients in whom a history of IVDU could be ruled out definitively helped to establish HIVAN as a distinct clinical entity (5,6).

In the 18 years since HIVAN was first described, much has been published regarding the epidemiology, pathogenesis, and treatment of this disease. Despite these advances, however, HIVAN continues to be an important cause of renal failure in the United States and other countries with large populations of African descent. Several excellent reviews on clinical aspects of HIVAN have recently been published (7–10). This article will focus on recent advances in our understanding of HIVAN epidemiology, pathogenesis, and treatment.

Definition of HIVAN

HIVAN is usually diagnosed in patients who have been HIV-1-seropositive for several years, and most patients have low CD4 counts and/or other criteria for the diagnosis of AIDS (11). In the post–highly active antiretroviral therapy (HAART) era, however, more patients are being identified who present earlier in the course of HIV infection with CD4 counts above 200 (12,13). HIVAN is characterized clinically by the presence of proteinuria, often but not necessarily in the nephrotic range (13,14). Most patients have moderate to severe renal insufficiency at the time of diagnosis (11,14,15), although a cohort of patients with HIVAN and mild renal insufficiency has been recently described (13).

The most common histopathologic abnormalities found in HIVAN include collapsing FSGS, microcystic dilation of renal tubules, lymphocytic interstitial infiltrates, and interstitial fibrosis (Figure 1) (16,17). The finding of collapsing FSGS, especially with coexistent tubular microcystic disease in an HIV-1-seropositive patient is diagnostic of HIVAN. Moreover, these pathologic findings in a patient who is not known to be HIV-1-seropositive should alert the physician to the possibility of the presence of HIV-1 infection in that patient.

In studies published in the pre-HAART era, endothelial tubuloreticular inclusions (TRI) were present in a large percentage of HIVAN biopsy specimens (16). In the post-HAART era, however, TRI are less commonly found (17). TRI are inducible by α-interferon exposure; therefore, the disappearance of TRI likely reflects the effectiveness of HAART in reducing systemic α-interferon levels (18).

A variety of renal diseases have been reported in HIV-1-seropositive patients. Even among seropositive black patients, although HIVAN is the most common finding at the time of renal biopsy, approximately 40% will have diagnoses other than HIVAN (17,19). Renal biopsy, therefore, is required to establish a definitive diagnosis of HIVAN.

Epidemiology of HIVAN in the Pre- and Post-HAART Era

During the 1980s and early 1990s, the incidence of end-stage renal disease (ESRD) due to HIVAN increased more rapidly than any other etiology of renal disease (20). In 1999, HIVAN became the third leading cause of ESRD in African Americans aged 20 to 64, and HIVAN is the leading cause of chronic renal failure in HIV-1-seropositive patients (19,21,22). Since the introduction of HAART, the incidence of death due to AIDS has decreased markedly in the United States for all ethnic groups, including African Americans (Figure 2) (23). The incidence of ESRD due to HIVAN has decreased much less rapidly, however (Figure 2). New cases of ESRD due to HIVAN (reported as AIDS nephropathy by the USRDS) increased rapidly until 1995 and then suddenly began to decrease slightly in 1996 (21) (presumably reflecting the effect of HAART). However, the rate of decline in the incidence of ESRD due to AIDS nephropathy has slowed; in 1999 (the most recent year for which data are available), the number of new cases actually increased (Figure 2).

The reported incidence of ESRD caused by HIVAN does not reflect the many patients with chronic renal failure and/or proteinuria due to HIVAN who have not yet reached ESRD and are thus not listed in the national databases. The prevalence of HIVAN among HIV-1-seropositive black patients has been estimated to be between as low as 3.5% in a cohort of HIV-1-seropositive patients screened for proteinuria in a primary care setting (24) and as high as 12% in a recent autopsy series (25). According to the Centers for Disease Control, there are
approximately 140,000 African Americans currently living with AIDS (23). These data suggest that there are between 4900 to 17,000 black patients in the United States with HIVAN. The prevalence of HIV-1 infection and AIDS are increasing in the United States, particularly among black patients (23). Of the 40 million people living with HIV/AIDS worldwide, 28.5 million reside in Sub-Saharan Africa (26). The incidence of HIVAN in Africa, however, is unknown. Assuming the prevalence of HIVAN among black patients in this region is similar to that for HIV-1–infected black patients in the United States, one would predict that there are between 1 and 3.4 million prevalent cases of HIVAN in Sub-Saharan Africa. It is likely that the lack of published literature on HIVAN in Africa is related to multiple factors, including a lack of surveillance and reporting for renal disease. HIVAN is usually a late manifestation of HIV infection (11); therefore, it is likely that many Africans with AIDS die of opportunistic infections early in the course of AIDS, before HIVAN becomes clinically evident. It is predictable that when medical care for HIV-infected Africans improves and patients live longer with AIDS, HIVAN will become an increasingly important cause of morbidity and mortality in Africa.

Recent data suggest that as mortality due to opportunistic infections is decreasing among patients with AIDS in the United States, other disease processes are becoming increasingly important in this patient population. A recent review of causes of death reported on death certificates of HIV-infected Africans revealed that, although some diseases such as wasting/cachexia and dementia/encephalopathy were decreasing in incidence, renal disease was increasingly reported (27). In this series, renal disease ranked fourth among conditions contributing to death in this patient population.

**Racial Predilection of HIVAN**

The marked racial predilection of HIVAN for black and Hispanic patients has been reported previously (19,28–30). Recent studies have confirmed this association. Recent data from the United States Renal Data System (USRDS) revealed that HIVAN is more strongly associated with black race than any other cause of renal failure with the exception of sickle cell disease (31). Hailemariam et al. (32) reported a series of 239 autopsies performed on patients with AIDS in Switzerland from 1981 to 1989 (before the introduction of HAART). Various renal abnormalities were reported among the 228 white patients. However, the only case of HIVAN in this series was detected in one of six African patients included in the study.

The marked racial disparity in HIVAN suggests genetic factors are important determinants of HIVAN pathogenesis. Nearly 25% of patients with HIVAN have first-degree or second-degree family members with ESRD, and black patients with HIVAN are 5.4 times more likely to have a first-degree or second-degree relative with ESRD than are black patients without renal disease (33).
The Duffy antigen/receptor for Chemokines (DARC) has been proposed as a candidate gene involved in HIVAN pathogenesis. The DARC promoter has a high prevalence of polymorphisms in black patients, and Liu et al. (34) have demonstrated increased DARC expression in renal specimens from children with HIVAN and hemolytic uremic syndrome. A subsequent study, however, failed to detect an association between DARC promoter polymorphisms and HIVAN (35).

**Pathogenesis of HIVAN**

**Role of HIV-1 Infection of Renal Epithelial Cells.**

Until recently, it was unknown whether HIV-1 infection of renal parenchymal cells caused HIVAN directly or whether HIVAN was an indirect renal response to HIV-induced immune dysregulation. The primary reason for this uncertainty was the presence of conflicting data regarding the presence of HIV-1 in renal parenchymal cells in clinical HIVAN specimens. In 1989, Cohen et al. (36) reported detection of HIV-1 in renal epithelial cells by DNA *in situ* hybridization. Other investigators reported detecting HIV-1 by PCR in tubules microdissected from HIVAN biopsies specimens (37). However, other groups disputed the presence of HIV-1 in renal parenchymal cells in HIVAN biopsy specimens (38,39).

Studies using an HIV-1 transgenic mouse model of HIVAN have provided important insight into HIVAN pathogenesis. Mice transgenic for a replication-defective HIV-1 construct lacking the gag and pol genes, expressed under control of the viral promoter (long terminal repeat or LTR), develop proteinuria, renal failure, and histologic renal disease identical to HIVAN (40,41). Bruggeman et al. (42) later demonstrated that the HIV-1 transgene is expressed in renal glomerular and tubular epithelial cells and that transgene expression in renal epithelial cells was required for the development of the HIVAN phenotype.

Further support for a role of direct infection of renal parenchymal cells in HIVAN pathogenesis was provided by a macaque model of HIV-induced renal disease (43–46). Stephens et al. (46) reported that passage of a chimeric simian-human immunodeficiency virus (SHIV) containing sequence from HIV-1 and the simian immunodeficiency virus (SIV) was capable of causing severe glomerulosclerosis and tubular disease. Infection with different strains of SHIV resulted in varying severity of renal disease, suggesting differences in viral strains mediated renal pathogenesis. Viral RNA was detected in the glomerular fractions of diseased animals; however, the RNA was not localized to a particular cell type within the glomerulus.

Accumulating data from animal models of HIVAN led to renewed attempts to determine definitively whether HIV-1 infects renal epithelial cells in HIVAN. In 2000, Bruggeman et al. (47) reported a series of 20 HIV-1–seropositive patients with renal disease who underwent renal biopsies. All but one of the patients were black or Hispanic, and 15 had HIVAN. In 11 of 15 patients with HIVAN, HIV-1 was detectable in renal epithelial cells by RNA *in situ* hybridization. In several samples, the presence of HIV-1 was confirmed using riboprobes specific for both the nef and gag genes and by DNA *in situ* hybridization. HIV-1 RNA was detected in renal tubular epithelial cells (Figure 4A), glomerular visceral and parietal epithelial cells (Figure 4B), and interstitial leukocytes. The pattern of HIV-1 infection of renal tubules is focal (Figure 4A) and may involve epithelial cells from multiple nephron segments, including proximal tubule, thick ascending loop of Henle, and collecting duct. The distribution of HIV-infection of renal tubules is similar to the pattern of microcystic tubular disease in HIVAN (48).

The mechanism by which HIV-1 gains entry into renal epithelial cells is unknown. CD4, the receptor for HIV-1, and CCR5 and CXCR4, the major co-receptors for HIV-1 are not expressed in most normal renal epithelial cells. Some authors have detected CD4 and the major co-receptors in cultured renal epithelial cells (49); however, no published studies have definitively demonstrated their expression *in vivo* (39,50). Sev-

---

**Figure 4.** *In situ* hybridization for HIV-1 mRNA in HIVAN. Adapted from Bruggeman et al. (47) with permission. (A) HIV-1 mRNA is detected in the cytoplasm of tubular epithelial cells. Tubular lumens (TL) are frequently filled with cellular casts (CC) or protein casts (PC). The CC result from apoptosis of infected epithelial cells that slough into the lumen, although some cells may have detached but remain viable. (B) HIV-1 mRNA is detected in podocytes (arrowheads), and parietal epithelial cells (arrows). G, glomerulus; US, urinary space. Magnifications: ×60 in A; ×200 in B.
eral other co-receptors for HIV-1 have been recently identified (51), but whether they are expressed in renal epithelial cells remains to be determined.

The constellation of collapsing focal glomerulosclerosis combined with extensive tubular microcystic disease was previously thought to be relatively specific to HIVAN. Markowitz et al. (52), however, recently reported a series of seven white HIV-negative patients who developed renal failure, proteinuria, and histopathologic disease identical to HIVAN after treatment with high-dose pamidronate. These findings suggest that high-dose pamidronate, like HIV-1, is capable of injuring glomerular and tubular epithelial cells, resulting in a phenotype that is similar to HIVAN. Moreover, it is now clear that kidneys of white patients are capable of producing the HIVAN phenotype when exposed to a particular epithelial toxin. It is not clear whether HIV-1 fails to cause HIVAN in white patients because it is unable to infect their renal epithelium or because HIV-1 infection of their renal epithelium is not as injurious as it is in black patients.

The Kidney as a Reservoir for HIV-1.

Infection of renal epithelial cells by HIV-1 has important implications for HIV-1–seropositive patients not only because it contributes to renal disease but also because the kidney may be an important reservoir for HIV-1. Bruggeman et al. (47) detected HIV-1 by both RNA in situ hybridization and DNA in situ PCR in three patients who had undetectable viral loads in peripheral blood samples. Moreover, Winston et al. (12) reported a patient who developed HIVAN in the setting of acute HIV-1 seroconversion. Proteinuria, renal failure, and histologic abnormalities improved dramatically after treatment with HAART. Despite an undetectable viral load in the peripheral blood while on HAART, the patient continued to express HIV-1 in renal epithelial cells as determined by RNA in situ hybridization. Thus, even in the face of an optimal virologic response to antiretroviral therapy and clinical remission of HIVAN, HIV-1 infection persisted in the renal epithelium and the virus remained transcriptionally active at a low level.

Marras et al. (53) isolated HIV-infected renal tubules from two patients with HIVAN using laser capture microdissection to characterize the HIV-1 quasi-species present in the renal tubular epithelium. HIV-1 envelope sequences were amplified from isolated tubules by PCR and sequenced. Phylogenetic analyses were performed on envelope sequences from renal tubular epithelial cells and peripheral blood mononuclear cells (PBMC) from the same patient. In each patient, there was variation in the HIV-1 envelope sequences present in the renal epithelium. As viral replication is required for viral evolution and sequence variation, this study provided direct evidence that the HIV-infected tubular epithelium in HIVAN is capable of supporting viral replication. Moreover, the quasi-species of HIV-1 present in renal epithelial cells clustered separately from sequences derived from the same patients’ PBMC, indicating that HIV-1 infection of tubular epithelial cells represents a viral compartment that is separate from the blood. Thus, the renal tubular epithelium is a reservoir for actively replicating HIV-1 and may support evolution of viral strains that differ significantly from virus present in a patient’s blood. It is not known whether the renal epithelial compartment is more likely to harbor drug-resistant HIV-1 strains or whether the renal epithelium is susceptible to currently available antiretroviral drugs.

Mapping the Genes Responsible for HIVAN Pathogenesis.

The HIV-1 genome consists of nine genes encoding fifteen proteins (Figure 5). Transgenic animal models and in vitro assays modeling the HIVAN phenotype have been used to map the HIV-1 genes responsible for HIVAN pathogenesis. The HIV-1 transgenic mouse model of HIVAN studied in our laboratory (40,41) and a recently published transgenic rat HIVAN model (54) expressing the same transgene lack the structural gag and pol genes. Thus both the rat and mouse models express just seven of the fifteen HIV-1 gene products. It is unlikely, therefore, that the gag or pol genes are required for HIVAN pathogenesis, although they may have an impact on disease phenotype or progression in man.

One of the pathologic hallmarks of HIVAN is focal glomerulosclerosis, often of the collapsing type (16). These collapsing lesions are associated with vigorous podocyte proliferation and loss of podocyte differentiation markers, including synaptopodin, podocalyxin, and WT-1 (55). These podocyte abnormalities have also been demonstrated in the HIV-1 transgenic mouse model of HIVAN (56). In vitro studies have demonstrated that podocytes derived from HIV-1 transgenic mice demonstrate a lack of contact inhibition and increased anchorage-independent growth in culture (57,58). Employing a series of scanning mutations in the original parental backbone that placed stop codons in each of the open reading frames as well as a series of monogenic HIV-1–gene constructs, Husain et al. (58) determined that nef is necessary and sufficient to cause most of the HIV-induced changes in podocyte cell biology in vitro. Conversely, inhibition of HIV-1 viral transcription using synthetic cyclin-dependent kinase-9 (CDK9) inhibitors to inhibit tat transactivation of the LTR inhibits podocyte proliferation and cause re-expression of podocyte differentiation markers in vitro (59).

Other animal models support an important role for nef in HIVAN pathogenesis. Hanna et al. (60) reported that mice transgenic for HIV-1 expressed under the control of the human CD4 regulatory sequences develop an AIDS-like illness as well as renal disease, although how closely the renal disease resembles HIVAN is unclear. They also generated several transgenic lines with mutations in one or more HIV genes and found that expression of nef was necessary and sufficient to produce their renal phenotype (61). It is not clear whether the transgene was expressed in renal epithelial cells, as is the case in HIVAN. The same group later extended this work by demonstrating that the renal phenotype in their model was ameliorated by mutating

![Figure 5. Organization of the HIV-1 genome.](image)
one of the nef SH3 binding domains. The authors postulated that nef exerts its pathogenic effect in the kidney, in part, via activation of src-family tyrosine kinases. Nef has previously been shown to be capable of binding Hck as well as several other src-family tyrosine kinases in vitro. To determine whether Hck was important in modulating the renal disease in the nef transgenic mice, they crossbred their transgenic mice with Hck knockout mice and found that the development of renal disease was delayed, suggesting Hck may play a role in the pathogenesis of HIV-induced renal disease. Other studies using mice that express nef derived from simian immunodeficiency virus (SIV) under the same CD4 promoter construct develop a similar renal phenotype (62). SIV nef has much lower affinity for Hck than HIV-1 nef (63,64); therefore, Hck is unlikely to be the only pathway responsible for the development of renal disease in their animal model.

Podocytes and tubular epithelial cells proliferate in vivo in HIVAN (42,55,65,66). Cyclin-dependent kinase (CDK) inhibitors regulate cell cycle through inhibiting cyclin-CDK complexes (66). Shankland et al. (66) found that expression of two CDK inhibitors, p27 and p57, were decreased in podocytes from HIVAN biopsies while expression of another CDK inhibitor, p21, was increased. These host genes may play an important role in mediating the increased epithelial proliferation present in HIVAN.

**Treatment**

Despite HIVAN becoming an important cause of renal failure in the United States, no prospective randomized controlled studies evaluating treatment modalities for HIVAN have been published. Unfortunately, most existing studies are retrospective and/or lack proper controls. The following discussion will focus on the best available evidence concerning the efficacy of antiretroviral medications, angiotensin-converting enzyme (ACE) inhibitors, and steroids in the treatment of HIVAN.

**Antiretrovirals.**

During the years of 1990 to 1995 (before the introduction of HAART), the number of new cases of ESRD caused by HIVAN rose by over 75% annually (21). After the introduction HAART in the United States, however, the rise in new cases of ESRD due to HIVAN ceased abruptly (Figure 2). It is likely that this change reflects the efficacy of HAART in either preventing HIVAN or slowing progression to end-stage renal failure in patients with HIVAN.

Several studies have evaluated the use of antiretroviral medications for the treatment of HIVAN. Ifudu et al. (67) studied 23 HIV-1–seropositive patients, 14 of whom had at least 2+ proteinuria. HIVAN was diagnosed in the five patients who underwent renal biopsy. All patients were offered treatment with zidovudine. None of the 15 patients who were compliant had deterioration of renal function after a mean follow-up of 20.4 mo. The eight patients who were not compliant with zidovudine treatment all progressed to ESRD (average serum creatinine less than 2 mg/dl). All patients were offered 10 mg/d fosinopril. After follow-up of 12 to 24 wk, renal function remained stable in the 12 patients who consented to fosinopril therapy; in the eight patients who refused fosinopril, serum creatinine increased from 1.4 to 6.4 mg/dl. Seven patients in the study were receiving monotherapy with nucleoside reverse transcriptase inhibitors. It is not clear how the effect of antiretrovirals may have affected the results of the study. Although the limitations of these studies are clear, the data are suggestive and further prospective studies should be done to define the optimal role for ACE inhibitors in the treatment of HIVAN.

**ACE Inhibitors.**

The effect of ACE inhibitors on HIVAN progression has also been studied. Kimmel et al. (72) reported an increase in renal survival associated with captopril usage in a retrospective case-control study of 18 patients with biopsy-proven HIVAN. Burns et al. (13) prospectively evaluated 20 patients with HIVAN and mild renal insufficiency (average serum creatinine less than 2 mg/dl). All patients were offered 10 mg/d fosinopril. After follow-up of 12 to 24 wk, renal function remained stable in the 12 patients who consented to fosinopril therapy; in the eight patients who refused fosinopril, serum creatinine increased from 1.4 to 6.4 mg/dl. Seven patients in the study were receiving monotherapy with nucleoside reverse transcriptase inhibitors. It is not clear how the effect of antiretrovirals may have affected the results of the study. Although the limitations of these studies are clear, the data are suggestive and further prospective studies should be done to define the optimal role for ACE inhibitors in the treatment of HIVAN.

**Prednisone.**

Prednisone has been found in several studies to be associated with reduced risk of progressive renal failure in patients with HIVAN. Smith et al. (73) reported an observational study of 20 patients with HIVAN who were treated with prednisone. Most patients had advanced renal failure and heavy proteinuria at the time of diagnosis. Seventeen of the patients experienced a decrease in serum creatinine and/or proteinuria after treatment with prednisone. However, several of the patients relapsed, requiring repeated courses of prednisone, 11 patients died during the study period, and 6 developed serious infectious complications while on prednisone. Seven patients were still free of dialysis at a mean of 25 wk of follow-up. Although
provocative, many of the patients in this study had a poor outcome, and the lack of controls precludes drawing conclusions regarding safety and efficacy. Another study, case-control by design, evaluated the outcome of 21 patients with HIVAN and advanced renal failure, 13 of whom were treated with prednisone (74). The odds ratio for progression to ESRD in prednisone-treated patients was 0.2, and only treatment with prednisone and initial serum creatinine were significantly associated with renal outcome after correcting for several clinical variables. There were, however, more infectious complications in the steroid-treated group, which the authors point out may be accounted for by the longer follow-up of that group. These findings are consistent with a retrospective cohort study from France of 108 patients with HIVAN, 15 of whom were treated with steroids, in which, treatment with steroids was associated with an odds ratio of 0.29 for the progression to ESRD (15).

The only study in the HAART era evaluating the efficacy of prednisone in patients with HIVAN was recently published by Szczech et al. (69). This retrospective cohort study evaluated outcomes in 19 patients with HIVAN and other HIV-related renal diseases. After multivariate analysis of several clinical variables, the association between prednisone and reduced rate of decline in creatinine clearance remained highly significant.

Despite the suggestion of a renal benefit in patients with HIVAN who received prednisone, we believe that until the efficacy of prednisone is validated in prospective controlled trials, the potential for increased risk of infectious complications should preclude its routine use in the treatment of HIVAN. Prednisone should be considered only for short-term therapy in patients with aggressive renal disease and no active infectious complications while HAART is being titrated to maximally suppress viral replication.

Prognosis

The data regarding prognosis for renal and patient survival after the diagnosis of HIVAN are biased by the fact that the majority of these patients are referred to nephrologists late in the course of their renal disease and HIV-1 infection (11). Patients with HIVAN who are not treated with antiretrovirals, ACE inhibitors, or prednisone, generally have a poor renal prognosis with a mean time to progression to ESRD of 1 to 3 mo (13,67,72). The renal prognosis in the HAART era is less well defined. Case reports of patients with mild renal insufficiency treated with antiretrovirals and/or ACE inhibitors have reported some patients who survived for years with stable renal function (12,13,70). Clinical variables associated with progression of renal failure include elevated serum creatinine (15,74,75), low CD4 count (15,75), high HIV-1 viral load (75), higher level of proteinuria (15), and previous antiretroviral therapy (15). Better prospective data examining the outcome of patients with HIVAN are needed before clinical variables can be accurately used to predict the prognosis of this patient population.

Conclusions

Since HIVAN was first described 18 years ago, it has become an important cause of renal failure among black patients. After decreasing slightly since the introduction of HAART, the incidence ESRD due of HIVAN in the United States is again increasing. Although data are lacking, the prevalence of HIVAN is probably highest in Africa, where it will likely emerge as a major cause of morbidity and mortality as the prognosis for AIDS survival improves.

HIV-1 infection of renal epithelial cells is an essential component of HIVAN pathogenesis. Renal epithelial cells are a newly identified viral reservoir and a separate replicating compartment distinct from blood. Significant progress has been made in understanding the pathogenesis of HIVAN, particularly in defining the viral genes necessary for causing renal disease. Far less is known concerning host factors contributing to HIVAN pathogenesis.

Prospective-controlled trials are needed to evaluate the efficacy and optimal use of currently available agents, including antiretrovirals, ACE inhibitors, and steroids. Continued research into the mechanisms by which HIV-1 causes renal disease should eventually yield novel therapies for the treatment of HIVAN and other forms of FSGS.

Acknowledgments

We thank Leslie Bruggeman for her help in the preparation of figures for this manuscript and Mary Klotman for comments and reading.

References


