

# Pathogenesis of HIV-Associated Nephropathy

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**Summary:** Human immunodeficiency virus-associated nephropathy (HIVAN) is a leading cause of end-stage renal disease in the HIV-1-seropositive population. HIVAN, which is characterized by heavy proteinuria and a rapid decline in renal function, is caused by infection and subsequent expression of viral genes in renal epithelial cells, although the exact mechanism of viral entry into these cells is unknown. The infected renal epithelium is a distinct compartment that supports the evolution of viral strains that may diverge from those found in the patient's blood. Research using animal models and in vitro studies has shown that *vpr* and *nef* are the HIV-1 genes most responsible for inducing the characteristic clinical and histopathologic syndrome of HIVAN. Dysregulation of several host factors, including mediators of inflammation, apoptosis, proliferation, transcription, and cell-cell interactions, are also critical factors in determining whether infection of the renal epithelium will lead to HIVAN. Additional research is required to delineate the mechanisms of HIVAN pathogenesis further so that more effective interventions can be implemented to prevent and treat this disease.

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It has been nearly 25 years since the first published case series describing the clinical and histopathologic syndrome of human immunodeficiency syndrome-associated nephropathy (HIVAN).<sup>1</sup> The classic clinical presentation of HIVAN is one of rapidly progressive azotemia associated with severe proteinuria, often in nephrotic range, and little or no peripheral edema (reviewed by Wyatt et al in this issue of *Seminars in Nephrology*, p. 513).<sup>2</sup> HIVAN may not be characterized simply as a glomerular or tubulointerstitial renal disease because of widespread abnormalities present in the renal parenchyma. Biopsy findings include focal glomerulosclerosis, often of the collapsing variant, with collapse of the glomerular tuft and proliferation and hypertrophy of podocytes, sometimes forming a cellular "pseudocrescent" in Bowman's space.<sup>3</sup> Tubulointerstitial disease is also a

prominent finding, and includes microcystic dilatation of renal tubules and pleiocytic inflammatory interstitial infiltrates and fibrosis.<sup>4,5</sup> Since its original description, the pathogenesis of HIVAN has been studied intensely by many investigators. In this review, we describe the current state of our understanding of the mechanisms by which host and viral factors interact to create the clinical and pathologic syndrome of HIVAN.

## HIV INFECTION OF THE KIDNEY

### HIV-Infected Renal Epithelium Is a Distinct Viral Compartment

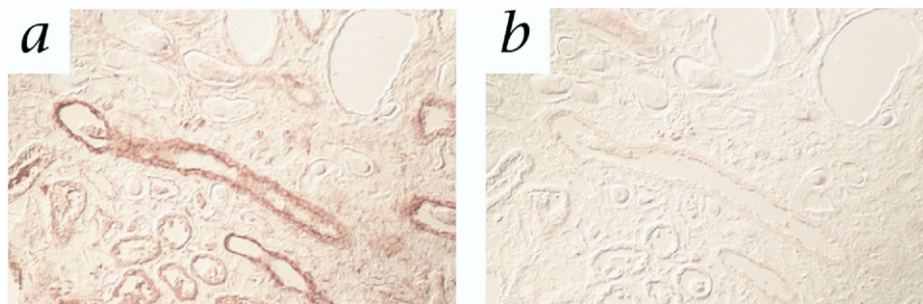
Several early studies focused on the question of whether HIV infects renal parenchymal cells in HIVAN with conflicting results.<sup>6-8</sup> The issue remained unresolved until Bruggeman et al<sup>9</sup> published a study in which biopsy specimens were collected prospectively from 21 HIV-positive patients with renal disease including HIVAN (N = 16) and other renal diseases (N = 5). The investigators used several techniques, RNA in situ hybridization, and DNA in situ polymerase

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**Figure 1.** Detection of viral nucleic acids in renal biopsy specimens. Detection of viral messenger RNA in HIVAN biopsy specimens by in situ hybridization. (a) Antisense riboprobe hybridization shows positive tubular epithelial cells. (b) A sense riboprobe was used as a negative hybridization control in serial section. Adapted by permission from Macmillan Publishers Ltd: *Nat Med.* 2002;8:522-526. Copyright 2002.<sup>13</sup>

chain reaction to detect HIV nucleic acid in renal biopsy specimens. They detected HIV infection of renal epithelial cells, including podocytes, glomerular parietal epithelial cells, and tubular cells, in the majority of biopsy samples. HIV was detected in kidney specimens from patients with HIVAN and other forms of renal disease and further studies have shown that HIV infection and tubular microcystic dilatation occur in a focal distribution that can affect all nephron segments.<sup>4</sup> Renal tubular infection by HIV-1 in a patient with HIVAN is shown in Fig. 1. These findings were confirmed recently and extended by Tanji et al,<sup>10</sup> who reported that in addition to renal epithelial cells, HIV-infected macrophages and T cells are present in the renal interstitium. No studies have definitely shown infection of nonepithelial renal parenchymal cells in vivo.

Interestingly, in the series from Bruggeman et al,<sup>9</sup> HIV-1 RNA was detected in each of the 4 patients who had no detectable viral RNA in the plasma, suggesting that HIV can remain transcriptionally active in renal epithelial cells even in the presence of maximal viral suppression with antiretroviral therapy (ART). This persistence of renal HIV expression was highlighted further by a report by Winston et al<sup>11</sup> of a patient who presented with HIVAN in the setting of acute HIV-1 seroconversion. Despite a rapid reduction of plasma HIV RNA to undetectable levels and resolution of clinical renal disease, examination of renal biopsy tissue taken after the response to treatment revealed no change in the expression of HIV-1 RNA. Together, these studies showed that HIV-1 infec-

tion of the renal epithelium occurs in the majority of HIV-infected patients with renal disease and that the virus is not eradicated from the kidney by currently available ART regimens.

The HIV-1 reverse transcriptase enzyme lacks a proofreading function and is therefore prone to making errors when synthesizing proviral DNA from its RNA template, allowing the virus to evolve rapidly and for distinct viral quasispecies to exist in each infected individual.<sup>12</sup> Marras et al<sup>13</sup> studied whether renal epithelial cells harbor HIV quasispecies that are distinct from blood-derived virus isolated from the same patients. They compared viral sequences isolated from HIV-infected renal tubules with those derived from peripheral blood mononuclear cells (PBMCs) from the same patients. They found that in each patient, the kidney-derived HIV sequences were similar to but distinct from the PBMC-derived HIV sequences. There are 2 important conclusions to be drawn from this study: the presence of distinct renal HIV quasispecies proved that renal tubular cells are capable of supporting HIV replication and that the kidney is a separate viral compartment that may support evolution of divergent viral strains. Because testing of blood-derived HIV for mutations that predict resistance to ART is an important strategy for guiding HIV treatment, it will be important to determine if the renal epithelium supports persistence of viral quasispecies that are resistant to ART.

### Mechanism of HIV-1 Renal Infection

The mechanism by which HIV-1 enters renal epithelial cells remains unknown. The virus in-

fects lymphocytes and macrophages via interaction of the viral envelope protein gp120 with the cellular CD4 receptor and either the CXCR4 or CCR5 co-receptor. Conaldi et al<sup>14</sup> detected CXCR4 and CD4 in a subpopulation of cultured renal epithelial cells, suggesting that HIV-1 may infect renal epithelial cells via these receptors. However, these studies were performed in cultured cells, which are vulnerable to contamination with nonepithelial cells and may express proteins not present in vivo. In contrast, Eitner et al<sup>8,15</sup> was unable to detect CXCR4 or CCR5 messenger RNA by in situ hybridization in renal epithelial cells in biopsy specimens from patients with HIVAN, normal kidneys, and patients with renal allograft rejection.

Ray et al<sup>16</sup> reported infection of primary renal tubular epithelial cells (RTECs) using HIV isolated from the PBMCs of children with HIVAN, but not when using common laboratory HIV isolates. The addition of a CD4 antibody did not inhibit infection, suggesting that RTEC infection by renal tropic HIV virus may occur via a CD4-independent mechanism. Another group characterized the tropism of chimeric viruses containing HIV envelope sequence cloned from infected tubular epithelial cells in HIVAN biopsy specimens and PBMCs from the same patients. They found that the kidney-derived viruses were capable of infecting cells expressing CD4 and either CXCR4 or CCR5, whereas the blood-derived viruses could infect only CCR5-expressing cells.<sup>17</sup> The investigators also showed that kidney-derived isolates were able to infect cell lines using the alternate HIV co-receptors BONZO/STRL33 and BOB/GPR15. This study suggests that the mechanism of viral entry into renal epithelial cells is dependent on both host and viral factors. However, because the in vivo expression of these alternate HIV co-receptors in renal epithelial cells has not been well characterized, their role in viral entry remains unknown. A recent study reported that the C-type lectin DEC-205 can mediate internalization and nonproductive infection of the HK-2-immortalized tubular cell line.<sup>18</sup> It is not clear how to reconcile this study with the many others showing that HIV establishes productive infection of tubular epithelial cells. Taken together, these studies suggest that

there may be renal tropic strains of HIV and the receptor use of these HIV variants may differ from common HIV isolates. However, the question of how HIV gains entry into renal epithelial cells remains unresolved.

### **HIVAN Is Caused by HIV Infection of Renal Epithelial Cells**

Studies using transgenic animal models have been invaluable for determining the viral and patient-related factors that contribute to the pathogenesis of HIVAN. In the most extensively used transgenic model, known as *Tg26*, mice are transgenic for an HIV provirus with deletions of the *gag* and *pol* genes that is expressed under control of the endogenous viral long terminal repeat (LTR) promoter. *Tg26* mice develop a clinical and histopathologic syndrome that is identical to HIVAN. Similar to human beings, in whom susceptibility to HIVAN is highly influenced by genetic factors (see the article by Wyatt et al in this issue, p. 513), the *Tg26* phenotype is highly dependent on the genetic background of the mice.<sup>19</sup> Gharavi et al<sup>20</sup> used genome-wide linkage analysis to identify genetic loci that are associated with a risk for developing the HIVAN phenotype, although the culprit genes have not yet been identified.

Investigators have used the *Tg26* model to determine whether the HIVAN phenotype is a result of renal expression of HIV-1 genes or, alternatively, the effect of systemic factors on the kidney. In reciprocal transplantation studies, kidneys from *Tg26* mice that were transplanted into wild-type mice developed the HIVAN phenotype whereas wild-type kidneys transplanted into *Tg26* mice remained normal, thereby showing that renal expression of HIV genes is necessary to produce the HIVAN phenotype.<sup>21</sup>

Expression of HIV-1 genes in vitro is able to recapitulate many of the cellular abnormalities associated with HIVAN in vivo. One of the histopathologic hallmarks of HIVAN is the presence of podocyte proliferation. Normal podocytes are terminally differentiated quiescent cells and in most forms of chronic renal disease the number of podocytes decreases.<sup>22</sup> In HIVAN, however, podocytes proliferate and undergo dedifferentiation with loss of expression

of podocyte-specific markers including CALLA, synaptopodin, WT-1, and podocalyxin.<sup>23,24</sup> Schwartz et al<sup>25</sup> showed that podocytes isolated from Tg26 mice have increased levels of proliferation and express lower levels of podocyte markers than podocytes isolated from wild-type mice. Furthermore, infection of wild-type podocytes with HIV-1 induces these same changes in vitro.<sup>26</sup> The availability of transgenic models and in vitro cell-based assays that reliably model the in vivo histopathologic features of HIVAN have allowed investigators to identify viral and host factors that are critical for HIVAN pathogenesis. The various transgenic models that have been used to study HIVAN pathogenesis are summarized in Table 1.

## VIRAL FACTORS

The polycistronic 9-kb HIV-1 RNA genome encodes 9 genes.<sup>27</sup> Animal models and in vitro studies have delineated the viral genes responsible for inducing derangements in the pheno-

type of renal epithelial cells and the mechanisms by which they produce renal pathology.

The Tg26 mouse model, which recapitulates the full HIVAN phenotype, lacks the *gag* and *pol* genes, making it unlikely that expression of either of these genes is necessary for HIVAN pathogenesis.<sup>19</sup> Moreover, Zhong et al<sup>28</sup> created several murine transgenic lines that expressed all HIV genes except *gag*, *pol*, and *env* under control of the *nephrin* promoter, thereby ensuring podocyte-specific gene expression. Most of these mice developed proteinuria and the typical glomerular and tubular changes of HIVAN. The renal phenotype in these mice also was dependent on the genetic strain of the mice, with a severe phenotype noted in FVB/N mice (same strain as Tg26) and minimal disease in C57BL/6 mice. This study suggests that expression of *vif*, *vpu*, *vpr*, *tat*, *rev*, and/or *nef* in podocytes is sufficient to induce the HIVAN phenotype. Several additional transgenic models expressing combinations of HIV genes un-

**Table 1. Summary of Transgenic Models of HIVAN**

Species/Reference	Promoter Controlling HIV Expression	HIV Genes Expressed	Location of Transgene Expression	Renal Phenotype
Mouse (Tg26) <sup>19</sup>	HIV LTR promoter	All HIV genes except <i>gag</i> and <i>pol</i>	Various tissues	FSGS with tubulointerstitial disease and proteinuria
Mouse <sup>28</sup>	<i>Nephrin</i> promoter	All HIV genes except <i>gag</i> , <i>pol</i> , and <i>env</i>	Podocytes	FSGS, tubulointerstitial disease, and proteinuria
Mouse <sup>31,32</sup>	CD4 promoter	Whole HIV genome as well as experimental mutations of individual HIV genes	CD4-positive cells	Tubulointerstitial disease (dependent on intact <i>nef</i> SH3 domain)
Mouse <sup>34</sup>	<i>Podocin</i> promoter-driven Cre recombinase activates <i>nef</i> expression	<i>nef</i>	Podocytes	Podocyte dedifferentiation and proliferation; no proteinuria or FSGS
Mouse <sup>23</sup>	HIV LTR promoter	All HIV genes except <i>gag</i> , <i>pol</i> , and <i>nef</i>	Various tissues	FSGS, microcystic tubular dilatation, interstitial infiltrate, proteinuria
Mouse <sup>35</sup>	HIV LTR promoter	Deletions of <i>vpr</i> and/or <i>nef</i>	Various tissues	FSGS, and proteinuria only in mice with intact <i>vpr</i>
Mouse <sup>35</sup>	<i>c-fms</i> promoter	<i>vpr</i>	Macrophages	FSGS with evidence of epithelial cell proliferation but no tubular disease
Mouse <sup>29</sup>	<i>Nephrin</i> promoter	Transgenic lines expressing individual HIV genes ( <i>vif</i> , <i>vpr</i> , <i>vpu</i> , <i>nef</i> , <i>rev</i> , or <i>tat</i> ), and <i>vpr/nef</i> double transgenic	Podocytes	Podocyte injury and FSGS in <i>vpr</i> and <i>nef</i> transgenic mice, worsened glomerular disease in <i>nef/vpr</i> mice
Rat <sup>74</sup>	HIV LTR promoter	All HIV genes except <i>gag</i> and <i>pol</i>	Various tissues	FSGS, mesangial hypercellularity, microcystic dilatation, and interstitial disease

Abbreviation: FSGS, focal segmental glomerulosclerosis.

der control of the HIV and/or cell type-specific promoters have been created to determine the gene(s) responsible for inducing HIVAN. Zuo et al<sup>29</sup> created transgenic mouse lines expressing a single HIV genes (*vif*, *vpr*, *vpu*, *nef*, *rev*, or *tat*) under the control of the *nephrin* promoter. Only transgenic mice expressing *vpr* and/or *nef* developed proteinuria and focal glomerulosclerosis, with the most severe phenotype occurring in mice transgenic for both *nef* and *vpr*. Accordingly, much recent research has centered on the role of these 2 genes in HIVAN pathogenesis.

## NEF

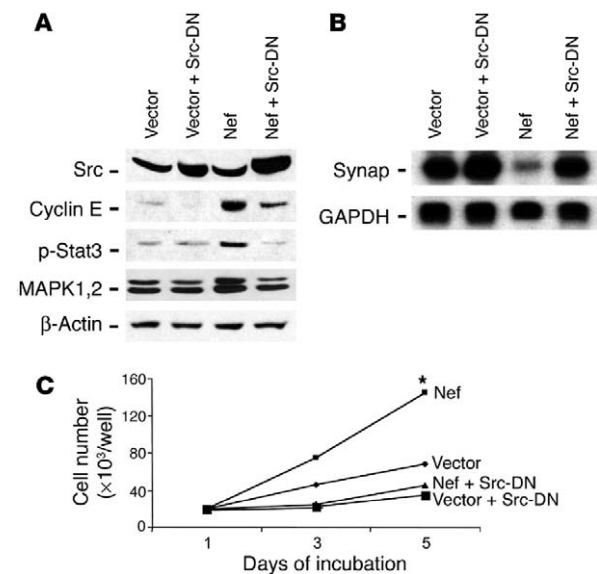
Nef is a 206-amino acid protein with many reported functions, including reduction of CD4 trafficking to the cell surface, effects on cytokine expression, and prevention of apoptosis.<sup>30</sup> Hanna et al<sup>31</sup> created transgenic mice that express *nef* under the control of the human CD4 promoter. These mice developed several features of acquired immune deficiency syndrome-like illness (loss of CD4<sup>+</sup> cells, wasting, and so forth) and interstitial nephritis. The relevance of this model to HIVAN is limited by the lack of a glomerular phenotype and because it is unlikely that the transgene was expressed in renal epithelial cells. When the same group later generated mice that were transgenic for *nef* with a mutated SH3 binding domain, the resulting mice developed neither an acquired immune deficiency syndrome phenotype nor renal disease.<sup>32</sup> This SH3 binding domain imbues Nef with the ability to interact with many proteins, including those of the Src-family of tyrosine kinases, which include Hck, Lck, and Fyn. Breeding CD4 *nef*-transgenic mice with *hck*-mutant mice did not prevent renal disease, suggesting that Nef-Hck interaction was not necessary for development of disease in this model.

To determine the HIV-1 gene(s) necessary for inducing podocyte proliferation, Husain et al<sup>26</sup> infected murine podocytes with a series of lentiviral vectors, each of which harbored a mutation in a single HIV gene. They found that mutation of *nef* completely eliminated podocyte proliferation and that infection of podocytes with a vector expressing *nef* alone induced podocyte

proliferation and dedifferentiation. *Nef* is therefore necessary and sufficient to induce podocyte abnormalities in vitro that closely model those found in HIVAN.

He et al<sup>33</sup> performed studies to elucidate the Nef-induced signaling pathways that result in the characteristic podocyte abnormalities in HIVAN. They showed that *nef* induced proliferation, and dedifferentiation of murine podocytes is mediated via Src kinase activation, with subsequent phosphorylation and activation of signal transducer and activator of transcription 3 (Stat3) and mitogen-activated protein kinase (MAPK)1,2. Higher levels of phosphorylated MAPK1,2 and Stat3 also were found in human HIVAN samples and Tg26 mice. Mutation of the Nef SH3 domain, which is required for its ability to interact with and activate Src kinase, prevented *nef*-induced proliferation and dedifferentiation. Conversely, expression of a dominant-negative Src also prevented the effects of *nef* expression (Fig. 2).<sup>33</sup>

Recently, 2 groups reported renal phenotypes in separate transgenic models in which



**Figure 2.** Role of Src activation in Nef-induced MAPK1,2 and Stat3 phosphorylation and phenotypic changes in podocytes. Control vector-infected (vector) or Nef-infected (Nef) podocytes were transfected with dominant-negative Src (src-DN) and then selected with hygromycin B. Inhibition of Src by Src-DN inhibited (A) Nef-induced activation of cyclin E, Stat3, and MAPK1,2, (B) as well as Nef-induced decrease in expression of synaptopodin and (C) Nef-induced podocyte proliferation.<sup>33</sup> Adapted with permission from He et al.<sup>33</sup>

mice express *nef* under the control of podocyte-specific promoters. In the study by Husain et al,<sup>34</sup> the investigators did not detect clinical renal disease (proteinuria or renal failure), but podocytes that expressed *nef* showed loss of expression of the podocyte differentiation markers WT1 and synaptopodin, and had increased expression of the proliferation marker Ki-67. Zuo et al<sup>29</sup> also created mice in which *nef* was expressed specifically in podocytes using a *nephrin* promoter. They found that the phenotype of the mice was variable, with 4 of 11 founder lines developing glomerular disease (predominantly focal and segmental glomerulosclerosis). Further characterization revealed decreased expression of synaptopodin in glomeruli of *nef*-transgenic mice.

Together, these studies provide compelling evidence that expression of *nef* in podocytes is an important component of HIVAN pathogenesis, with particular roles in inducing podocyte differentiation and proliferation. The effect of *nef* expression in tubular epithelial cells has not been well studied.

## VPR

Vpr is a 96-amino acid protein whose actions in the HIV life cycle include facilitating nuclear import of the HIV preintegration complex and transactivation of the viral LTR promoter. A role for *vpr* in HIVAN pathogenesis was first suggested by Dickie et al,<sup>35</sup> who created HIV transgenic mice that had mutations in either *nef* or *vpr*. They found that only mice with an intact *vpr* gene developed proteinuria and glomerulosclerosis. The investigators also created transgenic mice expressing *tat* and *vpr* under the control of the LTR promoter. These animals developed significant proteinuria and glomerulosclerosis. The renal phenotype was more severe when these mice were crossed with the *vpr*-mutant mice, suggesting that other HIV genes worsen the course of *vpr*-induced renal disease. Interestingly, another transgenic line in which *vpr* was expressed under control of the *c-fms* (macrophage-specific) promoter also developed modest proteinuria and glomerulosclerosis. The mechanism of renal pathogenesis in these mice is unclear, however, because free Vpr protein can directly transduce cells, it is

possible that macrophage-derived Vpr was taken up by renal epithelial cells, resulting in the renal phenotype.

Few studies have addressed the role of *vpr* in inducing tubular disease. Studies in nonrenal cells have shown that *vpr* can induce several cellular effects, including G2/M cell-cycle arrest and apoptosis.<sup>36</sup> Recently, Rosenstiel et al<sup>37</sup> showed that *vpr* expression in the HK-2 human proximal tubular epithelial cell line impaired cytokinesis and induced accumulation of multinucleated cells. Moreover, they found that RTEC in Tg26 mice and human HIVAN biopsies had increased levels of epithelial cell hypertrophy and multinucleation. These findings suggest that the in vitro abnormalities observed in *vpr*-expressing HK-2 cells also are present in HIVAN, thus providing important insights into the mechanism of tubulointerstitial disease in HIVAN.

## TAT and ENV

Tat is a 101-amino acid protein that, similar to Vpr, can be detected in the serum of infected patients. Tat is a critical activator of HIV transcription and can induce cellular changes including cytokine production and apoptosis.<sup>38</sup> Conaldi et al<sup>39</sup> reported that incubation of primary podocytes with Tat protein induces dose-dependent proliferation and loss of differentiation markers in vitro. Deciphering the relevance of these studies to disease pathogenesis is hampered by the fact that the podocytes were derived from Caucasian patients, who are not usually susceptible to HIVAN, and because several investigators have failed to detect renal disease when Tat is expressed alone in murine models. It is possible, however, that Tat may potentiate renal injury when present in addition to *nef* and *vpr*.

The HIV-1 *env* gene encodes the gp160 protein, which after proteolytic cleavage yields the gp120 and gp41 proteins. Gp120 is present on the surface of HIV virions and facilitates infection of target cells by interacting with CD4 and a co-receptor. One group has reported that gp120 can induce aberrant proliferation and apoptosis of human mesangial cells<sup>40</sup> and apoptosis of both tubular and glomerular epithelial cells.<sup>41,42</sup> Because HIV is not known to infect mesangial cells in vivo, and mice expressing the

*env* gene alone do not develop renal disease, the role of gp120 in the pathobiology HIVAN remains unclear.

## HOST FACTORS

Although HIV infection of the renal epithelium is a necessary step in HIVAN pathogenesis, only genetically susceptible persons respond to this infection by developing HIVAN. The host response to HIV infection is therefore a critical determinant in the pathobiology of HIVAN. Although genetic factors clearly contribute to the susceptibility of blacks to HIVAN, these factors remain unknown. However, several studies have identified features of the response of renal epithelial cells to HIV gene expression that contribute to the development of progressive renal disease. The major renal epithelial cellular processes affected by HIV infection that lead to the development of HIVAN are summarized in Fig. 3.

## Factors Promoting Apoptosis and Fibrogenesis

Increased apoptosis of tubular epithelial cells and interstitial fibrosis are common findings in many nephropathies, including HIVAN. Bodi et al<sup>43</sup> compared biopsy specimens from patients with HIVAN with HIV-seronegative patients with focal segmental glomerulosclerosis and found a substantially higher rate of tubular ep-

ithelial apoptosis. Interestingly, no increase in apoptosis was observed in HIVAN glomeruli.

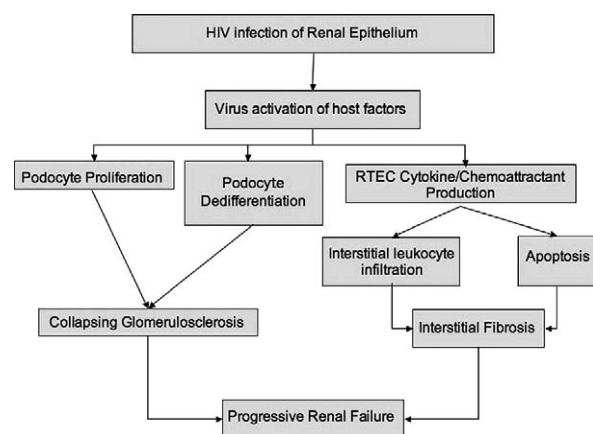
Conaldi et al<sup>14</sup> showed the ability of HIV-1 to infect human tubular cells and induce apoptosis in vitro. HIV infection induced extensive caspase-dependent apoptosis of proximal tubular cells and, in further experiments, the investigators found that the apoptosis-inducing receptor, Fas, was up-regulated by HIV infection. The importance of Fas up-regulation in HIV-induced tubular cell apoptosis was not clear because addition of Fas-blocking antibodies to the cells did not prevent apoptosis.

Transforming growth factor (TGF)- $\beta$  is a cytokine with important roles in promoting renal fibrogenesis and apoptosis in several animal models of renal disease.<sup>44-46</sup> Quantitative polymerase chain reaction analysis of biopsy specimens from patients with HIVAN showed significantly increased levels of TGF- $\beta$  messenger RNA when compared with biopsy specimens of HIV-positive patients without signs of HIVAN<sup>47</sup> and TGF- $\beta$  protein levels are increased in HIV-positive patients with glomerular disease.<sup>48</sup> In vitro cell culture models have suggested that HIV gene products may increase TGF- $\beta$  expression in renal parenchymal cells,<sup>49,50</sup> however, the effect of TGF- $\beta$  inhibition has not been tested in in vivo models of HIVAN.

Ross et al<sup>51</sup> reported that the ubiquitin-like protein *FAT10* is one of the most up-regulated genes after HIV infection in a RTEC line derived from a patient with HIVAN. Expression of *FAT10* also was increased in kidneys from Tg26 mice and in HIVAN biopsy specimens, and prevention of *FAT10* expression using RNA interference prevented HIV-induced apoptosis. Although these results suggest that *FAT10* expression is necessary for HIV-induced RTEC apoptosis,<sup>52</sup> the mechanism by which *FAT10* facilitates apoptosis is not known.

## Inflammatory Mediators

Tubulointerstitial inflammation is a prominent histopathologic finding in HIVAN.<sup>3</sup> Accordingly, several case series and retrospective case-control studies have reported an association between corticosteroid treatment and improved renal outcomes in patients of HIVAN.<sup>53-56</sup>



**Figure 3.** Diagrammatic depiction of biological pathways altered by HIV infection of renal epithelial cells, leading to progressive renal failure.

These observations suggest that renal inflammation is an important factor in the clinical course of HIVAN.

Ross et al<sup>51</sup> profiled the genes that were differentially expressed after infection of a human RTEC line derived from a patient with HIVAN. The most prominent response of the cells to HIV infection was up-regulation of proinflammatory mediators, including cytokines, chemokines, and adhesion molecules. Many of these same genes were found to be up-regulated markedly in kidneys from the Tg26 HIVAN model. These data suggest that expression of HIV genes in RTEC up-regulates proinflammatory genes that then recruit leukocytes to the kidney, resulting in the tubulointerstitial inflammation that is a characteristic finding in HIVAN.

In another study, investigators measured cytokine concentrations in the renal tissue of HIV-seropositive patients with and without HIVAN.<sup>57</sup> Several cytokines, including interleukin-8, monocyte chemoattractant protein-1, and RANTES, were increased in HIV-infected patients irrespective of whether they had renal disease. Patients with HIVAN were found to have higher renal tissue levels of major histocompatibility complex class II, interferon- $\gamma$  receptor, and interferon- $\alpha$ , although levels did not correlate with the amount of interstitial inflammatory cells. Together, these studies suggest that HIV-infected patients have increased renal levels of inflammatory mediators, which can recruit inflammatory cells into the interstitium.

## Proliferation

As discussed previously, the HIV-1 *nef* gene induces podocyte proliferation via activation of Src kinases and the transcription factors Stat3 and MAPK1,2.<sup>33</sup> Dysregulated expression of cyclins is also an important factor in promoting podocyte proliferation in HIVAN. HIV infection increases cyclin E expression in podocytes.<sup>58</sup> The expression of the cyclin-dependent kinase (CDK) inhibitors p27 and p57 are decreased in HIVAN biopsy samples as compared with normal kidneys or those with membranous and minimal change disease, whereas p21 levels are increased. Because CDK inhibitors suppress the activity of pro-proliferative cyclin/CDK com-

plexes, the increased levels of cyclin E, coupled with decreased p27 and p57, likely contribute to podocyte proliferation in HIVAN.

## Transcription Factors

Because the HIV genome does not encode an RNA polymerase, the virus must co-opt the host's transcriptional machinery to replicate itself. In lymphocytes, the transcription factors nuclear factor  $\kappa$ B (NF- $\kappa$ B) and Sp1 are important activators of HIV transcription, whereas in other cell types other transcription factors are more important. In studies using the Tg26 model of HIVAN, Bruggeman et al<sup>59</sup> determined that NF- $\kappa$ B and Sp1 regulate HIV expression in a manner similar to that seen in lymphocytes. In this model, there is constitutively increased phosphorylation and degradation of the NF- $\kappa$ B inhibitor, I $\kappa$ B $\alpha$ , freeing NF- $\kappa$ B to activate transcription of HIV and promote epithelial proliferation, apoptosis, and production of inflammatory mediators.<sup>60,61</sup>

CDK9 is a member of the protein complex that activates transcription of the HIV-1 LTR promoter in the presence of Tat protein.<sup>62</sup> CDK9 inhibitors inhibited expression of HIV and restored expression of differentiation markers in murine podocytes after infection with a *gag/pol*-deleted HIV virus.<sup>63</sup> The same group later showed that administration of CDK9 inhibitors to Tg26 mice prevented development of the HIVAN phenotype, suggesting that CDK9 inhibition may be a therapeutic strategy for the treatment and/or prevention of HIVAN.<sup>64</sup>

## Mediators of Cell Adhesion, Cell Signaling, and Extracellular Matrix

Kaufman et al<sup>65</sup> reported that the *sidekick-1* (*sdk-1*) gene is up-regulated in HIV-infected podocytes and levels of Sdk-1 protein are increased in vivo in podocytes in the Tg26 mouse model of HIVAN and in HIVAN biopsy samples. Sidekick proteins are members of the immunoglobulin superfamily and have roles in neuronal guidance during retinal development.<sup>66</sup> HIV-induced expression of SDK-1 leads to aggregation of podocytes in vitro and may contribute to the formation of podocyte "pseudocrescents" that are observed commonly in HIVAN.<sup>67</sup>



Podocan, a recently described small leucine rich protein, is up-regulated in sclerotic glomeruli of Tg26 HIV transgenic mice.<sup>68</sup> The role of Podocan in HIVAN pathogenesis remains speculative. Expression of another small leucine rich protein, Decorin, influences the course of glomerular disease in diabetic mice.<sup>69</sup> It is therefore plausible that levels of Podocan expression may affect the development of glomerulosclerosis in HIVAN.

Altered expression of vascular endothelial growth factor (VEGF) contributes to the pathogenesis of many renal diseases.<sup>70</sup> In mice, increased expression of the VEGF164 isoform induces a collapsing glomerulopathy similar to that found in HIVAN.<sup>71</sup> Korgaonkar et al<sup>72</sup> detected increased levels of VEGF protein and its transcriptional regulator (HIF-2 $\alpha$ ) in glomeruli in the murine HIVAN model and in human HIVAN biopsy specimens. Infection of murine podocytes with HIV in vitro induced expression of VEGF and HIF-2 $\alpha$ , which was mediated via *nef*-induced activation of Src kinase and Stat3. Moreover, addition of neutralizing antibodies against VEGFR2 reduced HIV-induced podocyte proliferation and dedifferentiation in vitro. These studies suggest an important role for VEGF in HIVAN pathogenesis.

## CONCLUSIONS

In susceptible individuals, HIV infection of renal epithelial cells leads to the development of HIVAN, which is characterized by histopathologic abnormalities that include collapsing glomerulosclerosis with tubulointerstitial disease. This renal syndrome is caused by the effects of HIV gene expression in the kidney, although the mechanism of HIV entry into renal epithelial cells remains unknown. The renal epithelium is a distinct viral compartment that supports active viral transcription even when the patient is treated with aggressive antiretroviral therapy.

Studies using transgenic murine models of HIVAN and in vitro studies have implicated *vpr* and *nef* as the HIV genes most responsible for inducing HIVAN. Host cellular responses to HIV infection are important determinants of renal disease and several cellular genes have been

implicated as contributors to the HIVAN phenotype.

The prevalence of persons living with end-stage renal disease as a result of HIVAN continues to increase even in countries where ART is widely available. Additional research is therefore urgently needed to delineate the pathogenesis of HIVAN and enable the design of more effective strategies for the prevention and treatment of this disease.

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