A novel HIV-1 transgenic rat model of childhood HIV-1–associated nephropathy

PATRICIO E. RAY, XUE-HUI LIU, LOUIS R. ROBINSON, WILLIAM REID, LIAN XU, JENNIE W. OWENS, ODELL D. JONES, FRANK DENARO, HARRY G. DAVIS, and JOSEPH L. BRYANT

Children’s Research Institute, Center for Genetic Medicine, Children’s National Medical Center, Washington, D.C.; The George Washington University, Washington, D.C.; Veteranarian Resources Program, Office of Research Services, National Institutes of Health, Bethesda, Maryland; and Animal Model Division, Institute of Human Virology, Biotechnology Institute, University of Maryland, Baltimore, Maryland


Background. A characteristic finding of human immunodeficiency virus (HIV)-associated nephropathy (HIVAN) is the presence of heavy proteinuria, focal or global glomerulosclerosis, and microcystic tubular dilatation leading to renal enlargement, and rapid progression to end-stage renal disease (ESRD) [1, 2]. Renal biopsy and autopsy studies showed the presence of large edematous kidneys, which contrasted with the small fibrotic kidneys typically seen in patients with chronic renal diseases of other etiology. In addition HIV-infected patients showed extensive glomerular capillary collapse and pronounced hypertrophy and hyperplasia of visceral epithelial cells associated with focal segmental and global sclerosis [1–3]. There was also severe tubulointerstitial injury and dilated tubules containing large casts, as well as frequent tubuloreticular inclusions in glomerular endothelial cells. This renal syndrome, termed HIV-associated nephropathy (HIVAN), was found predominately in HIV-infected African Americans [4, 5]. The presence of familial clustering of ESRD in African Americans with HIVAN suggests that these patients may have an inherited susceptibility to develop ESRD [6] when exposed to HIV-1.

Methods. We have recently developed the first HIV-1 transgenic rat model that carry a noninfectious HIV-1 DNA construct lacking 3.1 kb of sequence overlapping the gag and pol sequences, and develop many of the clinical lesions seen in HIV-infected patients, including HIVAN. To gain further insight into the pathogenesis of childhood HIVAN, we followed the clinical and renal pathologic outcome of 165 HIV-1 transgenic (HIV-Tg) rats and their respective control littermates for a period of 18 months.

Results. HIV-1 Tg rats progressively developed proteinuria and renal histologic lesions similar to those seen in children with HIVAN, leading to chronic renal failure. By in situ hybridization, HIV-1 genes were detected in glomerular and tubular epithelial cells and infiltrating mononuclear cells, which also expressed the HIV-1 envelop protein gp120. The development of HIVAN was associated with the accumulation of basic fibroblast growth factor (bFGF) in the kidney. Almost immediately after the first cases of HIVAN were reported, a controversy developed over the issue of whether the use of injected illegal drugs, or HIV-1 per se, were directly involved in the pathogenesis of this disease [7, 8]. Subsequent studies demonstrated HIVAN in children who acquired HIV-1 through vertical transmission [9–11], suggesting that HIV-1 infection per se was involved in the pathogenesis of HIVAN. In addition, HIV-1 transgenic (HIV-Tg) mice carrying a replication defective HIV-1 provirus were generated, and these animals developed a similar renal disease, even in the absence of immunosuppression and viral replication [12, 13].

Conclusion. These data support the notion that HIV-1 plays a direct role in the pathogenesis of HIVAN, by affecting the function and growth of renal epithelial cells, inducing the recruitment of mononuclear cells, and accumulating bFGF in the kidney, even in the absence of viral replication. These rats may provide an excellent model system to study the pathogenesis of childhood HIVAN.

In 1984, human immunodeficiency virus (HIV)-infected patients from New York and Miami presented with heavy proteinuria, and developed rapid progression to end-stage renal disease (ESRD) [1, 2]. Renal biopsy and autopsy studies showed the presence of large edematous kidneys, which contrasted with the small fibrotic kidneys typically seen in patients with chronic renal diseases of other etiology. In addition HIV-infected patients showed extensive glomerular capillary collapse and pronounced hypertrophy and hyperplasia of visceral epithelial cells associated with focal segmental and global sclerosis [1–3]. There was also severe tubulointerstitial injury and dilated tubules containing large casts, as well as frequent tubuloreticular inclusions in glomerular endothelial cells. This renal syndrome, termed HIV-associated nephropathy (HIVAN), was found predominately in HIV-infected African Americans [4, 5]. The presence of familial clustering of ESRD in African Americans with HIVAN suggests that these patients may have an inherited susceptibility to develop ESRD [6] when exposed to HIV-1.

Key words: childhood HIV-1 nephropathy, HIV-transgenic rats, basic FGF, captopril.

Received September 30, 2002
and in revised form November 22, 2002
Accepted for publication February 3, 2003
© 2003 by the International Society of Nephrology
genes/viral products throughout their gestation and postnatal periods, we are using HIV-Tg rodents to study the pathogenesis of childhood HIVAN. In the HIV-Tg mouse line, Tg6, we provided the first evidence that the expression of HIV-1 genes in tubular epithelial cells was associated with the development of renal enlargement and microcystic lesions characteristic of HIVAN [17]. In a follow-up study, we demonstrated that HIV-1 can infect primary human renal tubular epithelial cells derived from children with HIVAN [18]. Other studies have shown that HIV-1 can infect cultured adult human renal epithelial cells [19], detected HIV-1 RNA in glomerular and tubular epithelial cells in renal sections from adult patients with HIVAN [7, 20], and showed in vivo evidence of HIV-1 replication in renal epithelial cells [21]. Here, we describe for the first time the clinical and histologic outcome of HIVAN in HIV-Tg rats carrying a replication-defective HIV-1 provirus similar to the one introduced into HIV-Tg6 mice, and discuss the clinical relevance of this new model in the context of the pathogenesis of childhood HIVAN.

METHODS

HIV-Tg rats

Animal care was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The generation of the HIV-Tg rat model, and the overall description of the HIV-Tg (line 1), were described in detail in a previous publication [22]. Briefly, HIV-Tg rats were generated using a construct derived from the infectious HIV-1 provirus, pNL4-3, after deletion of a 3.1 kb Sphl-Bal1 fragment that overlaps the gag and pol genes (pNL4-3:dl443) [22]. Detection of the HIV-transgene was done by Southern blot analysis of tail DNA as previously described [22]. The HIV-Tg rats were derived from the Fisher 344/NHsd Sprague-Dawley background. Nontransgenic Fisher 344/NHsd Sprague-Dawley littermates were used as controls.

Experimental design

A total of 165 HIV-Tg rats (line 1), and their nontransgenic siblings were followed longitudinally from birth up to 18 months of age. Renal histology was done in 99 male and 66 female HIV-Tg rats. Seventy-four HIV-Tg rats (40 males and 34 females) were sacrificed randomly at different time points during the first 6 months of life, and before they showed clinical symptoms (8 to 12 rats at each time point). The remaining 91 HIV-Tg rats (58 males and 32 females) were sacrificed whenever they showed clinical signs of wasting, neurologic symptoms, pallor, or were found moribund in their cages. Control nontransgenic siblings of similar age and gender were sacrificed at identical time points (3 to 10 rats at each time point).

Definitions

HIV-Tg rats showing normal renal histology under light microscopy, and without abnormal proteinuria (<30 mg/dL) were considered without renal disease. An early clinical diagnosis of HIVAN was suspected by the presence of persistent and progressive proteinuria (>100 mg/dL). In this study, these rats are referred to as HIV-Tg rats with proteinuria, unless indicated otherwise. The diagnosis of HIVAN was confirmed by renal histology. Late HIVAN was defined clinically by the presence of persistent heavy proteinuria (>2000 mg/day) and/or elevated blood urea nitrogen (BUN) and creatinine values and confirmed by the presence of severe focal/global glomerulosclerosis, and generalized tubular dilatation with casts and microcysts. All these criteria were defined prior to the initiation of the study.

Biochemical analysis

Blood was collected to measure the hematocrit as described before [23]. Serum samples were collected in ice-cold tubes to measure BUN, creatinine, albumin, cholesterol, and triglycerides, using commercially available kits at the Animal Diagnostic Laboratory LLC (Baltimore, MD, USA). Hematuria and proteinuria were measured with commercially available test strips. Urine protein/creatinine ratios were measured with a colorimetric assay and the picric acid method for protein and creatinine values, respectively. Urinalysis and cast were observed by light microscopy in samples of unspun urine.

Blood pressure measurements

Systolic arterial blood pressure measurements were taken in conscious restrained rats using an automated system with a photoelectric tail-cuff [23]. After the rats were preconditioned to the chamber, the mean of three blood pressure readings was collected. The blood pressure was also measured in HIV-Tg rats with proteinuria (>30 mg/dL) but without renal failure (BUN <16 mg/dL), treated with 40 mg/L captopril (Bristol-Myers Squibb, Princeton, NJ, USA), or vehicle [phosphate-buffered saline (PBS)] in the drinking water (N = 4 in each group).

Morphologic analysis

Light microscopy. Kidneys from normal and HIV-Tg rats were fixed in 10% formalin and embedded in paraffin. Sections (4 μm) were stained either with hematoxylin and eosin, periodic acid-Schiff reagent (PAS), and Masson trichrome stain, following standard techniques. For electron microscopy, tissue was fixed in 2% gluteraldehyde, 2% paraformaldehyde in 0.1 mol/L cacodylate buffer (pH 7.2). Samples were postfixed in 1% OsO4 and infiltrated with propylene oxide resin. Thin sections were stained with uranyl acetate and lead acetate prior to photography.
HIV gene expression

Northern analysis. Kidneys from HIV-Tg rats of 7, 14, 30, and 60 days of age, respectively (five male rats in each group), were snap-frozen in liquid nitrogen and total RNA was isolated by using Trizol (Life Technologies, Gaithersburg, MD, USA). Total RNA (10 mg) was electrophoresed through 1% agarose/2.2 mol/L formaldehyde gels in 1 × 3-[N-morpholine] propane sulfonic acid (MOPS) and transferred to Nytran membranes (Schleicher and Schuell, Keane, NH, USA). RNA was ultraviolet cross-linked. Radioactive probes were prepared by the random prime method (Boehringer Mannheim, Indianapolis, IN, USA). To detect all species of the HIV transgene RNA, we used an HIV-1 nef cDNA probe obtained from the AIDS Repository and Reference Reagent Program, Division of AIDS, NIAIDs, NIH (pGM92, contributed by Dr. John Rossi), which overlaps the viral long terminal repeats (LTR). Blots were normalized by probing for the small ribosomal protein S14 (American Type Culture Collection, plasmid #59247). Blots were washed under stringent conditions at 55°C in 1 mol/L sodium phosphate, pH 7.4, 0.5 ethylenediaminetetraacetic acid (EDTA), and 10% sodium dodecyl sulfate (SDS) buffer and autoradiographed at 70°C. Membrane quantification was performed using a PhosphoImager (Molecular Dynamics, Sunnyvale, CA, USA), and the results expressed as a ratio of HIV transgene/S14 gene expression.

In situ hybridization. In situ hybridization (ISH) studies were performed as previously described [22], using digoxigenin-uridine triphosphate (UTP)-labeled RNA probe from Lofstrands Labs (Gaithersburg, MD, USA). We also used HIV-1 probes derived from the RNA expression plasmids pGM92 and pGM93, which were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (contributed by Dr. John Rossi). These plasmids contain in opposite orientation a 1.1 kb fragment from the 3’ end of the HIV-1 coding region that is common to all HIV-1 mRNA. The negative controls included (1) hybridization with the sense probe, (2) RNase A (100 mg/mL) in 10 mmol/L Tris HCl, pH 8.0,1 mmol/L EDTA) pretreatment before hybridization, and (3) omission of either the antisense RNA probe or the antidigoxigenin antibody. Reverse transcription polymerase chain reaction. Total RNA was extracted from normal and diseased kidneys using Trizol (Gibco, BRL, Grand Island, NY, USA). Reverse transcription (RT) of 2 μg of total RNA was performed using the Reverse Transcription Kit from Promega (Madison, WI, USA) as previously described [24]. The resulting first-strand cDNA was used as template for subsequent polymerase chain reaction (PCR) using the Advantage PCR Kit from Clontech (Palo Alto, CA, USA). The primers used to amplify the HIV-1 transgene were the following env specific primers: env-sense (5’-TGT GTA AAA TTA ACC CCA CTC TG-3’) and env antisense (5’-ACA ACT TGT CAA CTT ATA GCT GGT-3’). A 500 bp fragment of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was amplified as an internal control using specific mouse GAPDH primers. The PCR was performed at an annealing temperature of 58°C for 30 cycles for HIV-1 env and 25 cycles for GAPDH. The products were separated on 1.2% agarose gels and stained with ethidium bromide. In each experiment, distilled water was used as a negative control for contamination. For quantification, the amplified cDNA bands were measured by densitometry image scanning as described before [24], and the product yield was expressed as a ratio to GAPDH.

Immunohistochemistry studies

Frozen sections. Kidney tissues were snap-frozen in 22-oxacalcitriol (OCT) (Miles, Elkhart, IN, USA) using dry ice–chilled isopentane. Sections (5 μ thick) were brought to room temperature, fixed in cold acetone, and blocked with 5% normal goat serum, followed by incubation with the respective primary immunoglobulin (Ig) G, A, or M (Zymed, South San Francisco, CA, USA) and tetraacetic acid (EDTA), and 10% sodium dodecyl sulfate (SDS) buffer and autoradiographed at 70°C. Membrane quantification was performed using a PhosphoImager (Molecular Dynamics, Sunnyvale, CA, USA), and the results expressed as a ratio of HIV transgene/S14 gene expression.

Paraffin embedded sections. Paraffin sections were cut at 5 μm, deparaffinized, and rehydrated. Endogenous peroxidase activity was blocked by treating with 3% H2O2 in 100% methanol for 10 minutes. Immunostaining was performed with a commercial streptavidin-biotin-peroxidase complex Histostain™ SP kit (Zymed) according to the manufacturer’s instructions as previously described [23, 24]. In order to identify rat mononuclear cells, we used ED-1, a monoclonal IgG1 antibody against rat macrophages (Harlan, Bioproducts, Indianapolis, IN, USA). The HIV-1 protein, gp120, was detected with a gp120 antibody (ABL, Inc., Kensington, MD, USA). Double staining of ED-1 and the HIV-1 gp120 was done with the Histostain-DS™ double staining kit (Zymed). Vimentin expression was detected using a vimentin antibody from DAKO (Carpinteria, CA, USA). Cell proliferation was evaluated by using a biotinylated antiproliferative cell nuclear antigen (PCNA) monoclonal antibody (clone PC10). In selected sections, these experiments were confirmed with a rabbit antihuman Ki67 antibody from DAKO. Basic fibroblast growth factor (bFGF) was identified using affinity purified IgG fractions (2.5 μg/mL) from a rabbit polyclonal antibody directed against unique peptide sequence of bFGF (provided by Dr. A. Baird, PRIZM Pharmaceuticals, San Diego CA, USA) [25], and a murine monoclonal antibody against rh-bFGF DE6.
(provided by Dr. T. Reilly, Dupont-Merck, Wilmington, DE, USA) [26]. Controls included replacing the primary antibody with equivalent concentrations of nonimmune IgG and omitting the first antibodies. Analysis of the immunohistochemistry data was done using a computer-assisted image analysis software (Optimas version 6.2; Media Cybernetics, Silver Spring, MD, USA) and digitalized images. All sections were stained under identical conditions and compared independently by two investigators. The percentage of renal glomerular/medullar interstitial areas that stained positive for each antibody in 10 microscopic fields at 50× was measured. A semiquantitative scoring system (0 to 4) was used: 0 = 0% to 5%, 1 = 6% to 15%, 2 = 16% to 25%, 3 = 26% to 50%, and 4 = 51% to 100%. The number of PCNA-positive cells and macrophages (ED-1-positive cells/mm²) in renal glomeruli and medulla was also counted.

**Statistical analysis**

Results are expressed as the mean value ± SEM. Differences between two groups were compared by Student *t* test. When more than two means were compared, differences were measured by one-way analysis of variance (ANOVA) followed by multiple comparisons using the Student-Neuman-Keul’s test. The nonparametric Kruskal-Wallis test was used whenever the distribution of the data did not follow a Gaussian distribution. *P* values less than 0.05 were considered significant.

**RESULTS**

**HIV-Tg rat model**

By Southern blots analysis in DNA extracted from HIV-Tg rats (line 1), we confirmed our previous findings [22], by detecting a dominant 7.4 kb HIV-1 band corresponding to 20 to 25 transgene copy numbers, as estimated from the relative intensity of hybridization to Southern blots of transgene DNA compared to serial dilutions of known amounts of transgene DNA fragments (data not shown).

**Clinical and biochemical outcome**

Whereas all control nontransgenic littermates had normal life spans and lacked clinical, biochemical, and histopathologic evidence of ESRD throughout the course of the study, all HIV-Tg rats developed significant proteinuria before reaching 18 months of age, and approximately 80% developed ESRD by this time. In HIV-Tg rats with normal kidneys (~40 days of age), mean serum albumin was 4.4 ± 0.2 g/dL, serum cholesterol was 59 ± 4 mg/dL, and serum triglycerides was 147 ± 12 mg/dL. In contrast, in HIV-Tg rats of similar age with heavy proteinuria but normal renal function, serum albumin was 3.5 ± 0.1 g/dL, serum cholesterol was 104 ± 7 mg/dL, and serum triglycerides were 327 ± 12 mg/dL (mean ± SEM; *N* = 10 rats per group; *P* < 0.05 when compared to normal rats, respectively). Serum levels of cholesterol and triglycerides were further increased in HIV-Tg rats of similar age with chronic renal failure (data not shown). These rats also showed lower hematocrit values (35% ± 4%) when compared to HIV-Tg rats without renal disease (46% ± 3%) or their corresponding normal control littermates (47% ± 2%).

To evaluate the course of the renal disease, we measured the urinary protein excretion in control and HIV-Tg rats followed longitudinally from birth. At 7 days of age, significant proteinuria (>100 mg/dL) was detected in approximately 3% of the HIV-Tg rats. By 14 days of age, 10% of the HIV-Tg rats showed significant proteinuria, with protein/creatinine ratios of 13 ± 2 (mean ± SEM). Among 165 HIV-Tg rats, 32% developed ESRD before or at 100 days of life, and these animals showed urine protein/creatinine ratios of 65 ± 5 (mean ± SEM). In contrast, control rats of similar age showed protein values <30 mg/dL by urine dipstick or mean protein/creatinine ratios of 4 ± 1.6. Approximately 60% of the total number of HIV-Tg rats developed ESRD before or at 1 year of age, while none of the normal littersmates developed ESRD. These data however, may not accurately reflect the incidence of ESRD at 100 and 360 days, respectively, since many rats with and without symptoms were sacrificed before they reached these time points.

**Correlation between clinical and histologic findings**

The renal disease was detected earlier, and it was more severe in male HIV-Tg rats. Among the 74 HIV-Tg rats that were sacrificed randomly during the first 6 months of life (40 males and 34 females), uremia was detected most frequently in male rats (3:1 ratio). In addition, more HIV-Tg male rats developed ESRD within the first month of life (4 males vs. 1 female). Of interest, young HIV-Tg rats showed a faster rate of progression of the renal disease when compared to older rats. Approximately 70% of the HIV-Tg rats, in which proteinuria (>100 mg/dL) was detected during the first 20 days of life, developed ESRD within 25 days. In contrast, only 30% of the HIV-Tg rats, in which similar levels of proteinuria were detected at approximately 45 days of age, developed ESRD within a similar period of time (*N* = 10 rats in each group, *P* < 0.05).

Approximately 17% of the HIV-Tg rats without clinical symptoms, and sacrificed randomly during the first 8 weeks of life, showed histologic evidence of HIVAN. In contrast, 62% of the HIV-Tg rats sacrificed during the first 8 weeks of life due to the presence of clinical symptoms, showed histologic evidence of HIVAN. Overall, 84% of the HIV-Tg rats sacrificed during first 6 months of age due to the presence of clinical symptoms showed histologic evidence of HIVAN (100% males and 70% females). In HIV-Tg rats that developed clinical symp-
injury, medial thickening due to hypertrophy/hyperplasia of vascular smooth muscle cells, and the presence of perivascular inflammatory cells localized predominantly to the adventitia and interstitium surrounding the renal vessels (Fig. 1A, panel D). On gross inspection, the kidneys from HIV-Tg rats with chronic renal failure were diffusely pale and enlarged (35% ± 8% increased in weight when compared to kidneys from the littermate control rats). As shown in Figure 1B (panels A and B), the capsular surface of HIV-Tg kidneys with late renal disease was pitted, similar to that seen in humans with HIVAN. Some glomeruli showed evidence of capillary collapse, and others showed either segmental or global sclerosis (Figure 1B (panels C and D). By PAS and Masson trichrome staining, renal sections from HIV-Tg rats with late-stage renal disease showed an accumulation of extracellular matrix proteins in sclerotic glomeruli, renal interstitium, and around dilated tubules (Fig. 1B and 2). Glomerular parietal and visceral epithelial cells appeared reactive and enlarged. These kidneys also showed microcystic tubular dilatation with epithelial cell atrophy, attenuation and degeneration of the tubular epithelium. Some tubules were filled with PAS-positive casts. There was diffuse infiltration of mononuclear cells adjacent to dilated tubules and in glomeruli undergoing different stages of the renal disease. These mononuclear cells stained positive with an antibody against the HIV-1 protein gp120 and ED-1 (Fig. 3C).

Blood pressure

Healthy controls, HIV-Tg rats without renal disease, and HIV-Tg male rats with proteinuria but without chronic renal failure (~40 days old), showed mean normal systolic blood pressure levels of 103 ± 5 mm Hg, 101 ± 3 mm Hg, and 106 ± 4 mmHg (mean ± SEM), respectively (N = 10 rats in each group; P > 0.05). In contrast, HIV-Tg rats of similar age and gender (N = 5) but with chronic renal failure, showed higher systolic blood pressure levels, 143 ± 8 mm Hg (P < 0.05). Older HIV-Tg rats (~6 months of age) with proteinuria but without chronic renal failure also showed normal systolic blood pressure levels of 105 ± 6 mm Hg (N = 8). A detailed histologic examination of renal sections from 10 HIV-Tg rats with proteinuria without chronic renal failure and normal blood pressure confirmed the presence of focal segmental glomerulosclerosis in all rats, and renal arteriopathy in three rats.

Captopril treatment

HIV-Tg rats with proteinuria but without renal failure treated with captopril for 7 days (N = 4), showed a significant reduction in systolic blood pressure levels (102 ± 3 mm Hg vs. 80 ± 4 mm Hg) (mean ± SEM, before and after treatment respectively, P < 0.05). Captopril treatment did not significantly affect the systolic blood pressure in normal healthy control rats (data not shown). In HIV-Tg rats, captopril treatment decreased the urine protein/creatinine ratio (19 ± 4 vs. 6.7 ± 2) (mean ± SEM, before and after treatment, respectively, P < 0.05). In contrast, HIV-Tg rats with proteinuria but without renal failure, treated with vehicle for 7 days (N = 4), showed no significant changes in systolic blood pressure levels (100 ± 4 mm Hg vs. 106 ± 6 mm Hg) and urinary protein excretion (protein/creatinine, 8.6 ± 2 vs. 13 ± 4) (mean ± SEM) before and after treatment, respectively.

Renal pathology

On renal histology, glomerular enlargement with mesangial hyperplasia (Fig. 1A, panel B), mild tubular dilatation, and infiltration of mononuclear cells were common lesions seen during the early stages of the renal disease in young rats. Approximately 15% of HIV-Tg rats developed a renal arteriopathy characterized by endothelial injury, medial thickening due to hypertrophy/hyperplasia of vascular smooth muscle cells, and the presence of HIV-1 RNA

To determine the expression of HIV-1 mRNA throughout the process of rapid kidney growth, we extracted kidney RNA from HIV-Tg rats of 7, 14, 21, and 60 days of age, respectively (N = 5 rats per group). By Northern blot analysis, the single-spliced 4 kb proviral mRNA was the most abundant band detected in young rats (Fig. Ray et al: HIVAN in HIV-transgenic rats
Fig. 1A. A Renal disease in human immunodeficiency virus transgenic (HIV-Tg) rats. (A and C) A representative hematoxylin and eosin staining in glomeruli and renal vessels of HIV-Tg rats without renal disease. (B) A representative renal section of an HIV-Tg rat with mesangial hyperplasia, undergoing the early stages of the renal disease. The arrow points to a focal adhesion of a distal glomerular tuft to Bowman’s capsule. (D) A representative hematoxylin and eosin staining in an HIV-Tg rat with renal arteriopathy (original magnification ×350).

Fig. 1B. Late renal disease in human immunodeficiency virus transgenic (HIV-Tg) rats. (A and B) Macroscopic pictures of the kidneys of HIV-Tg rat with late renal disease. Note the pitted external and internal renal surfaces (original magnification ×4). A representative periodic acid-Schiff (PAS) staining of the renal cortex (C) and medulla (D) in sections of HIV-Tg rats with late renal disease. A representative PAS staining of the renal cortex (E) and medulla (F) in sections of HIV-Tg rats without renal disease. Increased PAS staining is detected in sclerotic glomeruli (C) and dilated tubules (D) of HIV-Tg rats with renal disease (original magnification ×100).

Fig. 2. Masson trichrome staining in renal sections from human immunodeficiency virus transgenic (HIV-Tg) rats with and without renal disease. Representative sections of the renal cortex (A) and medulla (C) of HIV-Tg rats without renal disease, and the renal cortex (B) and medulla (D) of HIV-Tg rats with late renal disease. Note the accumulation of extracellular matrix proteins (blue color) in renal glomeruli and tubulointerstitium in HIV-Tg rats with renal disease (B to D) (original magnification ×400).

Fig. 3. Immunohistochemistry staining for mononuclear cells (ED-1–positive cells) and gp120 in human immunodeficiency virus transgenic (HIV-Tg) rats with human immunodeficiency virus-associated nephropathy (HIVAN). (A) Few ED-1–positive cells in the renal cortex of an HIV-Tg rat without renal disease (original magnification ×250). (B) A diseased glomerulus of an HIV-Tg rat with late renal disease (original magnification ×350). (C) Recruitment of ED-1–positive cells (red stain) in the renal medulla of an HIV-Tg rat with late renal disease (original magnification ×250). (D) A representative colocalization immunohistochemistry staining of ED-1 (red stain) and gp120 (dark stain) in the renal medulla of an HIV-Tg rat with late renal disease (original magnification ×500). (E) shows gp120–positive cells (dark stain) in the renal medulla of an HIV-Tg rat with renal disease (original magnification ×500). (F) A renal section from the same rat incubated with a non immune control antibody (original magnification ×500).
Fig. 4. Transmission electron micrographs of renal sections of normal and diseased human immunodeficiency virus transgenic (HIV-Tg) rats. (A) A normal glomerular structure of a normal rat kidney. (B) A glomerulus of an HIV-Tg rat with late renal disease. Note the effacement of podocytes, visceral epithelial cell cytoplasmic vacuoles and protein reabsorption droplets, thickened basement membranes, and capillary collapse. (C) A normal renal tubular structure of a control rat, characterized by the presence of microvilli, mitochondria, lysosomes, and basal infolding of cell membranes. (D) A representative renal tubule of an HIV-Tg rat with renal disease. Note the absence of microvilli, scarcity of cellular organelles, degeneration of epithelial cells, and proteinaceous material in the luminal fluid (original magnification ×7000).

Older rats showed higher levels of the 7.4 kb and 2 kb proviral mRNA (Fig. 5A). By RT-PCR studies with RNA extracted from rats of similar age and gender, with and without renal disease (N = 6 rats in each group), we found a mean reduction in HIV-1 mRNA expression of approximately 85% in diseased HIV-Tg kidneys. Figure 5B shows the results of one representative experiment done in five HIV-Tg rats. By ISH, HIV-1 mRNA was detected in renal tubular and glomerular epithelial cells, but no in other renal cell types (Fig. 6). Interestingly, HIV-1 mRNA expression was decreased in glomeruli with severe focal segmental glomerulosclerosis (Fig. 6A), and was not diminished in dilated/microcystic renal tubules. Finally, by ISH studies, we failed to detect HIV-1 mRNA expression in renal vessels.

Immunohistology

Frozen renal sections from HIV-Tg rats with late renal disease revealed deposition of coarse granular aggregates of IgM, and occasionally IgG only in severely sclerotic glomeruli, but lesser amounts of IgA and C3 in the same structures (data not shown). Kidneys from normal littermates or from HIV-Tg rats without renal disease or during the early stages of it did not show evidence of immune deposits (data not shown). HIV-Tg rats with renal disease showed a significant recruitment of PCNA-positive cells both in renal glomeruli and medulla (Fig. 7). These findings were reproduced in selected renal sections with a Ki67 antibody (data not shown). The

Fig. 5. Analyses of total kidney RNA extracted from human immunodeficiency virus transgenic (HIV-Tg) rats. (A) Northern blot analysis of total kidney RNA extracted from HIV-Tg rats without renal disease. RNA samples were hybridized with a nef cDNA probe that hybridizes with all proviral mRNAs (upper panel) and with the S14 probe (lower panel) to demonstrate equal loading of RNA in each lane. Lane 1, RNA sample from HIV-Tg rats sacrificed at 14 days of age; lane 2, RNA sample from HIV-Tg rats sacrificed at 14 days of age; lane 3, RNA sample from HIV-Tg rats sacrificed at 21 days of age; and lane 4, RNA sample from HIV-Tg rats sacrificed at 60 days of age. In each group, total kidney RNA extracted from five different HIV-Tg male rats was pooled. Three HIV RNA size bands of approximately 7.4 kb, 4 kb, and 2 kb were detected. (B) Reverse transcription-polymerase chain reaction (RT-PCR) analysis of total kidney RNA extracted from HIV-Tg rats. Amplified products were resolved on 1% agarose gels and visualized with ethidium bromide staining. Primers were specific for a 201 bp fragment of HIV-1 env (upper panel) and a 500 bp fragment of the mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (lower panel). Molecular weight marker φX174RF/Hae III; lane 1, distilled water; lanes 2, 3, and 5, total kidney RNA extracted from HIV-Tg rats with late renal disease; lanes 4 and 6, total kidney RNA extracted from HIV-Tg rats without renal disease. A linear relationship between input RNA and HIV-RNA amplification during the early stages of it did not show evidence of was noted after 25 to 30 cycles of amplification for HIV-1 env and GAPDH, respectively.
DISCUSSION

HIVAN is an important cause of ESRD among young African Americans infected with HIV-1 [1–6]. Despite the significant advances made during the last years using different animal models of HIVAN [12, 13, 17, 27–30], the exact pathogenic mechanisms responsible for the development of HIVAN are not completely understood.

To gain further insight into the pathogenesis of childhood HIVAN, we followed the clinical and renal pathologic outcome of the first HIV-Tg rat model, focusing on the most relevant pathogenic features of childhood HIVAN. These HIV-Tg rats carry as a transgene a deleted HIV-1 provirus under the control of the viral LTR [22]. Here, we have shown that the expression of HIV-1 genes/viral proteins in circulating mononuclear cells and renal epithelial cells, as well as the accumulation of bFGF in the kidney, were associated with the development of clinical and renal histologic lesions similar to those seen in childhood HIVAN [9–11].

Previous studies in HIV-1 transgenic mice have provided a significant advance in our understanding of the pathogenesis of HIVAN [12–14, 17, 27]. In this study, we have used the only HIV-Tg rat line available, and therefore, it could be argued that the renal disease could be due to the interruption of a host gene. On the other hand, the lesions found in these HIV-Tg rats, resemble those seen in the HIV-1 transgenic mouse line Tg9 [12–14, 17], which carry approximately the same copy number.

Table 1. Comparison of immunohistochemistry scores between normal and human immunodeficiency virus (HIV)-transgenic (HIV-Tg) rats with HIV-associated nephropathy (HIVAN)

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>HIV-Tg rats with late renal disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS, glomerular interstitial staining hpf</td>
<td>1.2 ± 0.2</td>
<td>3.6 ± 0.16*</td>
</tr>
<tr>
<td>PAS, medullary interstitial staining hpf</td>
<td>1.7 ± 0.2</td>
<td>3.8 ± 0.2*</td>
</tr>
<tr>
<td>Masson trichrome, glomerular interstitial staining hpf</td>
<td>1.4 ± 0.16</td>
<td>3.4 ± 0.16*</td>
</tr>
<tr>
<td>Masson trichrome, medullary interstitial staining hpf</td>
<td>1.6 ± 0.3</td>
<td>3.7 ± 0.15*</td>
</tr>
<tr>
<td>Vimentin staining in glomerular cells hpf</td>
<td>3.9 ± 0.1</td>
<td>2.7 ± 0.2*</td>
</tr>
<tr>
<td>Vimentin staining in medullar epithelial cells hpf</td>
<td>0.2 ± 0.1</td>
<td>3.8 ± 0.13*</td>
</tr>
<tr>
<td>bFGF staining in glomerular hpf</td>
<td>1.3 ± 0.15</td>
<td>3.4 ± 0.3*</td>
</tr>
<tr>
<td>bFGF staining in renal medulla hpf</td>
<td>1.9 ± 0.3</td>
<td>3.4 ± 0.2*</td>
</tr>
<tr>
<td>PCNA-positive cells in renal glomeruli cells/mm²</td>
<td>2.0 ± 0.3</td>
<td>10.0 ± 1.2*</td>
</tr>
<tr>
<td>PCNA-positive cells in renal medulla cells/mm²</td>
<td>10.0 ± 1</td>
<td>79.0 ± 2*</td>
</tr>
<tr>
<td>Glomerular macrophages ED-1-positive cells/mm²</td>
<td>1.2 ± 0.2</td>
<td>18.0 ± 0.9*</td>
</tr>
<tr>
<td>Medullary macrophages ED-1 positive cells/mm²</td>
<td>6.0 ± 0.8</td>
<td>45.0 ± 5*</td>
</tr>
</tbody>
</table>

Abbreviations are: PAS, periodic acid-Schiff; hpf, high power field; bFGF, basic fibroblast growth factor; PCNA, proliferating cell nuclear antigen. Mean ± SEM. The following semiquantitative scoring system was used: 0 = 0% to 5% cells or interstitial area stained; 1 = 6% to 15%; 2 = 16% to 25%; 3 = 26% to 50%; and 4 = 51% to 100%. The number of PCNA and macrophages (ED-1-positive) cells/mm² was counted both in renal glomeruli and medulla. *P < 0.05. N = 10 male rats per group (~40 days of age).
of an identical HIV-1 transgene. In both transgenic models, the viral LTR regulates the expression of the transgene, and epithelial cells are the intrinsic renal cells expressing HIV-1 genes. Three sizes of viral transcripts are detected in the kidney of HIV-Tg mice [12–14] and rats. These findings suggest that rev, a viral protein that regulates splicing can function both in rats and mice. In addition, the HIV-1 envelope protein gp120 is present in the circulation and produced by mononuclear cells of HIV-Tg rats [22], while gp120 is not detected in the circulation or mononuclear cells in HIV-Tg mice [12, 13]. Earlier reports on cultured human endothelial cells, have shown that gp120 can induce cytotoxic effects through a CXC chemokine receptor 4 (CXCR-4–mediated mechanism independently of the CD4 receptor [31, 32]. In addition, some HIV-1 strains can interact with the rat homolog of CXCR-4 [33], and the envelop protein induces in vivo neurotoxicity in mice [34] and rats through a similar mechanism [35]. Moreover, immunization of HIV-Tg mice with recombinant HIV-1 gp160 prolonged the survival and improved the outcome of their renal disease [36]. Taken together, these observations suggest that gp120 may play a role in the pathogenesis of the renal disease in HIV-Tg rats. At the present time, however, the mechanisms responsible for the activation of the HIV-1 transgene, in rat mononuclear cells are not clearly understood. The HIV-LTR is transactivated by the viral Tat protein, which requires cellular cyclin T as a cofactor [37]. Thus, it is possible that the rat cyclin T may have a more functional interaction with Tat in rat lymphoid cells. Overall, the presence of HIVAN in two different transgenic rodent models that carry the same HIV-1 transgene and the expression of HIV-1 genes/proteins in rat mononuclear cells strongly suggest that the renal disease in HIV-Tg rats is induced by HIV-1.

Despite the similarities between both HIV-Tg models, we have identified some interesting clinical and histologic features in HIV-Tg rats that are clinically relevant for studying the pathogenesis of childhood HIVAN. First, HIV-Tg rats develop mesangial hyperplasia during the early stages of the renal disease (Fig. 1A) and this is a typical lesion seen in children with HIVAN [9–11]. We have failed to detect HIV-1 mRNA in mesangial cells of HIV-Tg rats and HIV-infected children with renal disease [38]. Thus, this model could be used to study alternative mechanisms by HIV-1 induces mesangial hyperplasia in children. Second, the absence of edema and hypertension during the early stages of HIVAN, and the observation that captopril treatment induced hypotension in previously normotensive HIV-Tg rats with proteinuria, suggest that their intravascular volume status may be slightly contracted. In a similar manner, HIV-
infected children with proteinuria frequently develop intravascular volume contraction whenever their fluid intake is limited or undergo fluid electrolyte losses due to intercurrent illness. Children have a higher rate of basal heat production per kilogram of body weight than adults, and their metabolic, fluids, and electrolyte needs increase considerably during an acute illness and periods of catch-up growth. Therefore, these HIV-Tg rats could be used to study the pathogenesis of fluid electrolytes and growth-related problems of HIV-infected children. Third, HIV-Tg rats that developed proteinuria during the first 2 to 3 weeks of life showed a rapid progression to ESRD, suggesting that the developing rat kidney is very sensitive to the adverse effects of HIV genes/viral products. In contrast, older HIV-Tg rats underwent longer periods of proteinuria before they developed ESRD. These findings resemble the clinical situation seen in HIV-infected children, in whom both short and long periods of heavy proteinuria may precede the development of ESRD [9–11]. Finally, the accumulation of bFGF in the renal cortex and medulla of HIV-Tg rats with renal disease also mimics the situation of HIV-infected children with renal disease [24, 38].

Other findings in HIV-Tg rats may be relevant to study the pathogenesis of HIVAN in both children and adults. For example, one issue explored for the first time in HIV-Tg rats was to determine whether changes in blood pressure contributed to the pathogenesis of the renal disease and vascular arteriopathy. To the best of our knowledge, this issue has not been studied in other HIV-Tg mouse models. HIV-Tg rats with heavy proteinuria but normal renal function showed normal systolic blood pressure levels, suggesting that hypertension per se does not play a major role in the initial stages of HIVAN and/or development of the renal arteriopathy. These findings validate the HIV-Tg rat animal model system, since hypertension is not a major factor modulating the outcome/progression of the renal disease during the early stages in patients with HIVAN. Moreover, HIV-Tg rats, unlike HIV-Tg56 mice, do not develop edema or ascitis throughout the progression of the renal disease. In a similar manner, many HIV-infected children and adults do not show signs of edema despite the presence of heavy proteinuria. Taken together, these observations may explain the lack of hypertension typically seen in HIV-infected patients with heavy proteinuria and normal renal function. In contrast, during the late stages of the renal disease, HIV-Tg rats developed systolic hypertension, which is probably related to the expansion of the extracellular fluid volume associated with ESRD. Overall, these data suggest that captopril treatment could at least potentially improve the clinical outcome of HIVAN by reducing the proteinuria and improving the control of blood pressure during the early and late stages of HIVAN, respectively.

The renal histologic lesions in HIV-Tg rats are also strikingly similar to those found in HIV-infected patients [1–3, 9–11]. Focal segmental or global sclerosis, collapsing glomerulopathy, microcysts, and tubulointerstitial lesions leading to renal enlargement are typically seen in both children and adults with HIVAN. In HIV-Tg rats, proteinuria was detected as early as the first week of life, and always preceded the development of the renal histologic lesions. The appearance of proteinuria as the initial clinical sign of HIVAN may be at least partially explained by the induction of podocyte injury by HIV-1 genes/viral proteins [7, 39, 40]. On the other hand, the renal arteriopathy in HIV-Tg rats was not associated with the expression of HIV-1 mRNA in vascular endothelial or smooth muscle cells and could be detected even in rats without hypertension. Tinkle et al [41], using a different HIV-Tg mouse model, described a vasculopathy induced by the expression of HIV-1 mRNA in vascular smooth muscle cells and the recruitment of inflammatory cells in the adventitia. They suggested that FGFs released by smooth muscle and inflammatory cells may contribute to the pathogenesis of these lesions. In support of this notion, we have found an accumulation of inflammatory cells and bFGF in diseased HIV-Tg rat kidneys. However, more studies will be needed to elucidate the basic mechanism by which HIV-1 induces renal arteriopathy. Taken together, these findings suggest that HIV-1 may induce the development of renal lesions in HIV-Tg rats by injuring intrinsic renal cells and releasing vireal proteins and cytokines.

A typical finding in HIVAN is the presence of renal enlargement and formation of renal microcysts. We have shown that an excessive proliferation of renal tubular epithelial cells is at least partially responsible for these changes [17]. However, it is not clear whether HIV-1 gene products, per se, cytokines, or a combination of both factors, are responsible for these proliferative changes. Here, we have detected an overall reduction in the steady-state levels of HIV-1 mRNA in diseased HIV-Tg rat kidneys. These findings may be due to the generalized renal tubular injury, since epithelial cells are the predominant intrinsic renal cells expressing HIV-1 mRNA. However, by ISH studies, we found a robust expression of HIV-1 mRNA in epithelial cells surrounding the renal microcysts. Thus, it could be argued that HIV-1 genes might be directly responsible for the proliferation of renal epithelial cells [40]. Alternatively, cytokines released by injured renal cells may also induce the proliferation and up-regulate the expression of HIV-1 mRNA in mononuclear cells [42] or regeneration of epithelial cells. In support of this notion, the changes in vimentin expression in renal glomerular and tubular epithelial cells suggest these cells are undergoing active regenerative changes. In addition, regenerating tubular epithelial cells isolated from the urine of children with HIVAN
express high levels of bFGF and are sensitive to the growth promoting effects of bFGF [38]. Moreover, we have found a significant accumulation of bFGF in renal glomeruli and tubulointerstitium of children with HIVAN [24], and HIV-Tg rats show similar findings. Overall, the accumulation of bFGF in glomeruli and tubulointerstitium of HIV-Tg rats and children with HIVAN may contribute to the pathogenesis of the renal disease by changing the permeability of renal capillaries [43, 44], affecting the growth of podocytes [45, 46], and inducing the proliferation of renal epithelial [17, 24], mesangial [38], and vascular smooth muscle cells [26, 41].

CONCLUSION

When interpreted in the context of previous studies, our data support the notion that HIV-1 plays a direct role in the pathogenesis of HIVAN, at least partially by affecting the growth and differentiation of renal glomerular and tubular epithelial cells and by inducing the recruitment of mononuclear cells and the accumulation of bFGF in the kidney. Hopefully, these rats will provide a valuable model system to study the pathogenesis of HIVAN and to test novel therapies to prevent the progression of this disease.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health grants 2RO-1 DK 49419 and 2RO-1 HL 55605 (PER); and NIH grant DK 49419-S1 (LRR) 1 K08 A101792; and the “Fundación Argentina para el Desarrollo Infantil” from Buenos Aires, Argentina.

Reprint requests to Patricio E. Ray, M.D., Room R-211, Children’s Research Institute, Children’s National Medical Center, 111 Michigan Ave. N.W., Washington, D.C., 20010.

E-mail: Pray@cnm.org

REFERENCES

31. ULLREICH CK, GROPMAN JE, GANHU RK: HIV-1 gp120 and gp160-
induced apoptosis in cultured endothelial cells is mediated by caspases. *Blood* 96:1438–1442, 2000


37. BIENIAZ PD, GIRDON T, BOGERD HP, CULEN BR: Recruitment of a protein complex containing Tat and cyclin T1 to TAR governs the species specificity of HIV-1 Tat. *EMBO J* 23:7056–7065, 1998


