Focal glomerulosclerosis in proviral and c-fms transgenic mice links Vpr expression to HIV-associated nephropathy

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Abstract

Clinical and morphologic features of human immunodeficiency virus (HIV)-associated nephropathy (HIVAN), such as proteinuria, sclerosing glomerulopathy, tubular degeneration, and interstitial disease, have been modeled in mice bearing an HIV proviral transgene rendered noninfectious through a deletion in\(gag/po\). Exploring the genetic basis of HIVAN, HIV transgenic mice bearing mutations in either or both of the accessory genes\(nef\) and\(vpr\) were created. Proteinuria and focal glomerulosclerosis (FGS) only developed in mice with an intact\(vpr\) gene. Transgenic mice bearing a simplified proviral DNA (encoding only Tat and Vpr) developed renal disease characterized by FGS in which Vpr protein was localized to glomerular and tubular epithelia by immunohistochemistry. The dual transgenic progeny of HIV[Tat/Vpr] mice bred to HIV[ΔVpr] proviral transgenic mice displayed a more severe nephropathy with no apparent increase in Vpr expression, implying that multiple viral genes contribute to HIVAN. However, the unique contribution of macrophage-specific Vpr expression in the development of glomerular disease was underscored by the induction of FGS in multiple murine lines bearing a c-fms/vpr transgene.

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Introduction

Vpr protein is an accessory gene product of human immunodeficiency virus type 1 (HIV-1) exhibiting pleiotropic roles in the replicative cycle of the virus and on host gene expression. Vpr is packaged in the virion particle (Lu et al., 1993) and has an early role in the infection of nondividing cells by facilitating the nuclear transport of the virion core (Heinzinger et al., 1994; Hrimech et al., 1999). Vpr is synthesized late in the replication cycle and can induce terminal differentiation in producing cells (Levy et al., 1993), cell death (Planelles et al., 1995; Stewart et al., 1997), alterations in host cell activation (Kino et al., 1999; Kulkosky et al., 1999), and viral transcription (Bukrinsky and Adzhubei, 1999; Gummuluru and Emerman, 1999). A late function may be to boost virus production and transmission through the induction of G2 arrest (Jowett et al., 1995; Trono, 1995; Yao et al., 1998). The latter effect may be mediated by interactions with the transcriptional factor TFIIB (Agostini et al., 1999) or changes in the cytoskeletal structure of the host cell (Gu et al., 1997; Matarrese et al., 2000). Despite diverse cellular effects, there has been surprisingly little evidence of Vpr-related cytotoxicity in vivo. Dispensable in vitro for virus replication and cytotoxicity in T cells (Trono, 1995), it is also dispensable for T cell loss in a model of HIV infection (Aldrovandi and Zack, 1996) and AIDS in a model of SIV infection (Baba et al., 1999; Gibbs et al., 1995). Alternatively, Vpr may have a unique function in cells of the macrophage lineage where a positive effect on virus replication has been demonstrated (Balliet et al., 1994; Connor et al., 1995; Sherman et al., 2003).

Models of chronic HIV-1 infection exist in mice transgenic for proviral sequences. These have been useful for studying proviral gene activation in macrophages (Dickie et al., 2001; Gazzinelli et al., 1996; Schito et al., 2001).
Reactivation of virus in long-lived macrophages may be an important factor contributing to the persistent infection of humans (Crowe, 1995). We have assessed the toxicity of Vpr in transgenic animals carrying noninfectious HIV proviral DNA (gag and pol sequences were deleted). The vpr gene was genetically manipulated and its toxicity evaluated in the context of a nephropathy previously described in HIVΔGag/Pol mice (Dickie et al., 1991). These mice model a disease remarkably similar to HIV-associated nephropathy (HIVAN) in humans (Barisoni et al., 2000; Bruggeman et al., 1997; Dickie et al., 1991). Exploring a role for macrophages (or macrophage-like cells) in the etiology of HIVAN, Vpr expression was also linked to the macrophage-specific murine exon 2 c-fms promoter in novel transgenic mouse lines.

Renal disease has been associated with other immuno-deficiency viruses such as the feline- (Poli et al., 1993) and simian-associated (Alpers et al., 1997) viruses. In humans, nephropathy of diverse forms has been described, including sclerosing and immune-complex mediated forms of glomerulopathy and tubulointerstitial diseases. On the basis of the appearance of unique clinical features, HIV-associated nephropathy was recognized as the most prevalent form of kidney disease in HIV-infected populations (D’Agati and Appel, 1998). It affects a significant proportion of seropositive individuals (up to 10% of infected individuals, depending on the study), most notably amongst African-Americans and cases of pediatric AIDS. The principle histopathological lesion in humans is collapsing focal glomerulosclerosis (FGS) associated with podocyte hypertrophy, diffuse mesangial hyperplasia, and extracellular matrix deposition (D’Agati and Appel, 1998). Tubulointerstitial disease is also prevalent, marked by tubular epithelial cell simplification, tubular atrophy, microcystic dilatation, and inflammation. Despite the episodic detection of viral DNA and antigen in renal biopsies (Cohen et al., 1989; Green et al., 1992; Kimmel et al., 1993), and the in vitro infection of various renal cell types (Green et al., 1992; Ray et al., 1997), it remains unclear what renal cell types are permissive for HIV-1 replication in vivo. Coreceptor expression on both mesangial and tubular epithelial cell types has been proposed (Conaldi et al., 1998; Eitner et al., 1998) and tissue macrophages are likely to reside in the glomerular space (Sasmono et al., 2003). Cytopathic viral proteins may act directly on one or more of these glomerulus-associated cells (Bodl et al., 1995; Conaldi, 1998), or they may act by disrupting the cytokine milieu of the kidney (Rappaport et al., 1994).

HIVAN in transgenic mice (Dickie et al., 1991) featured glomerular and tubular defects in common with human HIVAN. Transplantation experiments implicated renal production of HIV gene products (Bruggeman et al., 1997) in the disease. Inactivation of nef did not prevent glomerular disease in one proviral transgenic line studied (Kajiyama et al., 2000), an observation independently made in transgenic mice expressing Nef under the transcriptional control of the human CD4 promoter (Hanna et al., 1998). We reported herein, on a similar approach, to evaluate the toxicity of HIV-1 Vpr on renal epithelia.

**Results**

**Derivation and characterization of HIV proviral transgenic lines**

The prototype HIV-1 transgenic mouse line (line T26) that developed HIVAN bore a ΔGag/Pol HIV-1 proviral DNA (pEVd1443 sequences originally derived from clone pNL4-3) (Dickie et al., 1991). Inactivation of the nef gene in an independent HIVΔd1443 transgenic line (X5 mice) mitigated but did not prevent the development of murine HIVAN (Dickie, 2000; Kajiyama et al., 2000). A pathologic role for HIV-1 Vpr was explored through the creation of novel HIV transgenic lines in which the vpr gene of pEVd1443 and its ΔNef counterpart pX5 was mutated by frameshifting at the unique EcoRI restriction site (base position 5743; Fig. 1). Two new HIV-1 transgenic lines were produced: V13 (ΔVpr) and VN15 (ΔVprΔNef). For the purpose of complementing these lines with vpr, pEVd1443 was modified by sequential deletion of vif, vpu, env, nef, and rev, leaving only the genes for Tat and Vpr intact (HIV[Tat/Vpr] transgene; Fig. 1). A second vpr orf was inserted in the 3′ position once occupied by nef to enhance the likelihood of Vpr expression.

Line V13 was one of six HIVΔd1443[ΔVpr] lines screened for viral gene expression by Northern analysis. V13 mice produced HIV transcripts in the skin, eye, and muscle with much reduced levels appearing in the kidney and lymphoid tissues (Fig. 2). V13 transgenic mice displayed perinatal, nuclear cataracts (absent the microphthalmia characteristic of T26 wild-type mice; Dickie, 1996) and, unique to our hemizygous HIV lines, were runted relative to nontransgenic littermates. Viral gene expression was not detected in tissues of the remaining five HIVΔd1443[ΔVpr] lines, and all were phenotypically normal. Line VN15 was one of eight HIVΔd1443[ΔVprΔNef] lines analyzed. Viral gene transcription was observed in four of the derived lines, predominantly in the skin. The highest level of expression was observed in line VN15. VN15 animals also expressed HIV RNA in the eye (Fig. 2). Hemizygous VN15 mice were visibly normal but homozygous offspring of this line (>30 observed) developed hyperkeratosis and necrotic lesions on the tail as did homozygous T26 mice (Santoro et al., 1994) and infectious proviral HIV-1 transgenic mice (Leonard et al., 1988).

Seven HIV[Tat/Vpr] mouse lines were analyzed. HIV transcripts were detected by Northern analysis in only one (line TV2). TV2 transgenic mice expressed viral RNA in the...
eye (Fig. 2) in this limited screen. These mice developed normally to adulthood and were visibly indistinguishable from nontransgenic littermates. However, nursing TV2 mothers (2–3 months of age) became moribund (13/13 mice, mean of 12 days post-partum) with signs of severe subcutaneous edema typical of wild-type HIVd1443 transgenic (T26) mice with renal failure. Homozygous offspring of TV2 crosses (male or female) became moribund around 3 months of age also with signs of subcutaneous edema (8/8; mean age of 105 days). In contrast, the incidence of morbidity in hemizygous TV2 mice (males and virgin females) was around 5% (3/59) for animals under 6 months of age.

**HIV gene expression in HIV[Tat/Vpr] kidneys**

Failing to detect Vpr protein in TV2 renal tissue by Western analysis, RT-PCR was performed on kidney RNA using the primer pair 045/RV1 (see Materials and methods) to detect spliced transcripts for Tat (338 and 411 bp), Vpr (725 bp), and full-length transcripts (at 1272 bp). The sizes of amplified cDNA sequences were predicted from splicing reactions for the parental NL4-3 virus (Purcell and Martin, 1993). Viral transcripts were detected in kidneys of 1- and 3-month-old hemizygous TV2 mice (Fig. 3). An
increase in HIV mRNA expression was observed in homozygous TV2 progeny relative to hemizygous sibling mice. This increase was apparent for putative vpr transcripts and the putative tat transcripts but not full-length HIV transcripts. Full-length transcripts were increased in nursing females relative to age-matched, virgin sibling mice, concurrent with modest increases in transcripts related to vpr and tat genes based on predicted product sizes. Neither gene dosage nor the physiologic conditions peculiar to nursing mice appeared to alter the ratio of the two small transcripts.

Ultrastructural changes and Vpr deposition in TV2 renal tissue

Characteristic features of HIV-associated nephropathy were observed by electron microscopy in sections of 6-month-old TV2 kidneys: podocytes displayed foot process effacement, capillary lumen were collapsed, basement membranes were focally thickened and wrinkled, and there was microvillous transformation (Figs. 4A, B). Previously, HIV-related protein was detected immunologically in glomeruli and tubular epithelia of T26 and X5 transgenic mice (Dickie et al., 1991; Kajiyama et al., 2000). Similarly, TV2 kidney sections were screened with a polyclonal antibody raised against a peptide fragment of Vpr (residues 1–46). Though Vpr was not detectable in kidneys of virgin hemizygous TV2 animals, it was detected in kidneys of lactating hemizygous TV2 females (Figs. 4C, D). Vpr was localized to the renal cortex, near apical regions or brush borders of the occasional proximal tubular epithelial cell. Staining of the occasional glomeruli for Vpr protein followed a segmental pattern. This tissue localization was consistent with endogenous expression in glomerular epithelia, the trapping of Vpr within sclerotic glomeruli, or its reabsorption by tubular epithelial cells.

Vpr gene expression in kidneys of HIVd1443 mice

By RT-PCR, levels of vpr expression were compared in wild-type HIVd1443 (T26) mice, ΔNef and ΔVpr counterparts (X5, V13, and VN15), and TV2 mice (Fig. 5). Mutant transcripts of vpr were distinguished from wild-type transcripts based on the susceptibility of amplified cDNA products to EcoRI cleavage. Vpr sequences (633 bp product amplified with primer pair L2/VH2) were prominent in T26 mice, less so in X5 mice and TV2 mice. Wild-type vpr transcript was not observed in either V13 or VN15 mice (Fig. 5B). When these latter lines were crossed with TV2 mice, the observed level of native vpr transcript was similar to that observed in TV2 mice alone (Fig. 5B). Likewise, no significant change was observed in the expression of the 3′ vpr gene of TV2 (screened using primer pair LV1/R3, Fig. 5C) in TV2-complemented HIVd1443 mice.

The apparent decrease in vpr expression in X5 (ΔNef) mice relative to wild-type T26 mice, when total HIV gene
expression was elevated (Fig. 2), suggested that viral gene splicing was different in X5 mice. Splicing differences were probed by screening for Rev-encoded products at 136, 142, and 160 bp; Env at 120 and 136 bp; Tat at 319 bp; and Nef at 120 bp, the sizes of which were predicted from known viral splicing products (Purcell and Martin, 1993). HIVd1443 (T26) kidney RNA yielded predominant fragments of 120 bp (corresponding to putative Env or Nef transcripts) and 633 bp (for Vpr) (Fig. 5D). By comparison, X5(DNef) RNA yielded less vpr-specific transcript, consistent with experiments shown in Fig. 5C. The inactivation of vpr (in V13 mice) was associated with reduced levels of 120–140 base pair products, consistent with either nef or env transcripts (Fig. 5D). The most striking difference in VN15 (ΔNefΔVpr) mice was an enhanced level of RT-PCR product near 320 base pairs (the putative tat product) that was absent in animals inheriting the TV2 transgene.

Characterization of c-fms/vpr transgenic mice

Given the propensity of HIV-1 provirus transgenic mice to express viral genes in macrophages and the presence of macrophages in renal glomeruli and tubules (Sasmono et al., 2003), we assessed the renal toxicity of Vpr in mice transgenic for a c-fms/vpr gene. The exon-2 c-fms promoter is specific for macrophages (Himes et al., 2001). The M-CSF receptor may also be expressed in mesangial cells of renal glomeruli, a cell type with macrophage-like characteristics (Watanabe et al., 2001). Founder mice and heterozygous offspring bearing the c-fms/vpr transgene (Fig. 1) were visibly normal. In two of three lines analyzed (MeRV4 and MeRV21), transgenic mothers displayed a transient edema between 12 and 15 days of lactation. Unlike TV2 mothers, however, MeRV females survived to produce multiple litters. Females in the third MeRV line (MeRV8) nursed without complication.
Renal expression of vpr in MeRV mice was screened by RT-PCR using a primer pair nested in the vpr gene (LV1/RV1). Heterozygous female mice from the three MeRV lines were compared to TV2 mice (Fig. 6). Each of the three MeRV lines expressed the vpr gene in the kidney, the highest levels appearing in MeRV21 animals. The level of vpr expression in each was consistently less than that observed in TV2 mice.

Renal dysfunction in association with the vpr gene

Renal expression of vpr in MeRV mice was screened by RT-PCR using a primer pair nested in the vpr gene (LV1/RV1). Heterozygous female mice from the three MeRV lines were compared to TV2 mice (Fig. 6). Each of the three MeRV lines expressed the vpr gene in the kidney, the highest levels appearing in MeRV21 animals. The level of vpr expression in each was consistently less than that observed in TV2 mice.

Urine protein levels in V13 mice were actually subnormal, perhaps reflecting the poor growth characteristics of these mice. Amongst the HIV[Tat/Vpr] lines, only the TV2 line displayed elevated protein excretion. Hemizygous TV2 mice at 5–8 weeks of age displayed modestly elevated levels of urine protein (Fig. 7A), but changes did not match the significance of T26 or X5 animals (Table 1). By comparison, high urine protein levels distinguished homozygous TV2 mice (3× control values). Lactating female TV2 mice at 10 days post-partum displayed urine protein levels well above normal (1220 ± 230 mg/dl vs. 260 ± 140 mg/dl for nontransgenic nursing mice; n = 7). Thus, increased levels of proteinuria mirrored the increased morbidity in TV2 mice.

The development of proteinuria and morbidity in TV2 hemi- and homozygous mice, coupled with the absence of renal disease in the Tat-positive, Vpr-deficient HIVd1443
transgenic lines, implicated Vpr in the induction of murine HIVAN. Exploring a role for secondary viral factors, and in particular Nef, Vpr-deficient V13 and VN15 mice were complemented with vpr through breeding with TV2 mice.

Table 1
Hemizygous HIV transgenic lines and their susceptibility to HIV-associated nephropathy (HIVAN)

<table>
<thead>
<tr>
<th>Transgenotype (encoded genes)</th>
<th>Lines expressing HIV</th>
<th>Established line</th>
<th>Urine protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIVd1443 (vpu, vpr, tat, nef, env)</td>
<td>4/8</td>
<td>T26</td>
<td>2155 ± 250</td>
</tr>
<tr>
<td>HIVd1443[ΔNef] (vpu, vpr, tat, env)</td>
<td>1/7</td>
<td>X5</td>
<td>1370 ± 514</td>
</tr>
<tr>
<td>HIVd1443[ΔVpr] (vpu, tat, nef, env)</td>
<td>1/6</td>
<td>V13</td>
<td>150 ± 40</td>
</tr>
<tr>
<td>HIVd1443[ΔVprΔNef] (vpu, tat, env)</td>
<td>4/8</td>
<td>VN15</td>
<td>265 ± 65</td>
</tr>
<tr>
<td>HIV[Tat/Vpr] (tat, vpr)</td>
<td>1/7</td>
<td>TV2</td>
<td>390 ± 120</td>
</tr>
</tbody>
</table>

HIV genes present in each line are shown in italics. The fraction of founder lines that expressed viral genes as detected by Northern analysis is shown. Urine protein levels are presented for HIV transgenic females at 35 days of age (n = 12). Control FVB/N mice had a urine protein level of 310 ± 100 mg/dl at this age. Variability is given as one standard deviation. The elevation in TV2 urine protein (relative to nontransgenic controls) was not significant (to P < 0.01).

Fig. 7. Proteinuria in HIV proviral transgenic mice. Urine protein levels (A) in hemizygous TV2 mice (n = 12) are compared to nontransgenic siblings (FVB/N, n = 12) and mice homozygous for the transgene (TV2/TV2, n = 4). In B, the hemizygous offspring of V13 × TV2 breeding pairs were analyzed for urine protein (TV2, n = 2; V13, n = 3; V13/TV2 dual transgenic offspring, n = 3). Eight hemizygous T26 mice (●) and five VN15/TV2 mice (▲) are included for comparison. Average urine protein values for transgenic mice are presented, after normalization to urine protein levels in nontransgenic siblings (for example, T26 mice had urine protein levels 4-fold greater than nontransgenic siblings at 21 days of age). Error bars have been omitted for clarity (see Table 1 for typical error values). TV2 (●); V13 (▲); TV2/TV2 (●).

VN15/TV2 dual transgenic mice were indistinguishable from sibling mice bearing only the TV2 transgene in that they developed a mild proteinuria (Fig. 7B) with no associated morbidity. In contrast, urine protein levels in V13/TV2 mice were elevated almost threefold relative to nontransgenic sibling mice (Fig. 7B) and 6 times that of their runted V13 transgenic siblings. V13/TV2 mice died at the mean age of 40 days (5/5 mice).

C-fms/vpr (MeRV) mice displayed no proteinuria up to 6 months of age. Renal function in MeRV mice was assessed from urine protein-to-creatinine (P/C) ratios (Fig. 8). Lower creatinine concentrations were observed in urine, contributing to elevated urine P/C ratios in the three MeRV transgenic lines. This indication of renal dysfunction in 2- to 4-month-old females was surprisingly consistent for all Vpr-positive transgenic lines. The exception was T26 mice. Moribund T26 mice had P/C ratios near 100, whereas surviving females had a mean P/C of 20 (n = 12).
Focal glomerulosclerosis as a defining feature of Vpr-related renal disease

The primary histopathological lesion observed in young TV2 hemizygous animals was solidification of glomerular tufts in a focal and segmental distribution (Fig. 9). In nursing TV2 mice, disease was marked by glomerular epithelial cell hyperplasia, adhesions to Bowman’s capsule, diffuse glomerulosclerosis, marked thickening of Bowman’s capsule, and periglomerular fibrosis (TV2*, Fig. 9). The tubular compartment contained atrophied cells and cells containing protein reabsorption droplets. Tubules were dilated and, in some cases, proteinaceous casts were present. The interstitium contained the occasional perivascular mononuclear infiltrate. Neither hemizygous V13 (ΔVpr) kidneys nor VN15 (ΔVprΔNef) kidneys displayed these or other signs of renal disease (not shown). The kidneys of 1-month-old V13/TV2 double-transgenic mice displayed widespread microcystic dilatation and FGS (Fig. 9) quite distinct from the subtle changes observed in TV2 mice of the same age. The histopathological condition of VN15/TV2 kidneys was indistinguishable from TV2 kidneys of age-matched siblings (not shown).

Consistent in the histopathological analyses of MeRV kidneys was the appearance of focal glomerulosclerosis (FGS) in both lactating and nonlactating females (Fig. 9) in the absence of tubular changes. All three MeRV lines displayed FGS with evidence of epithelial cell proliferation, reduced urinary space, and adhesions between glomerular tufts and Bowman’s capsule. The most severely affected animals were lactating females (MeRV21*, Fig. 9) where glomeruli appeared hypocellular and solidification was more global.

Discussion

A genetic analysis of HIVd1443 proviral transgenic mice was undertaken to explore the role of HIV-1 Vpr in the development of HIV-associated nephropathy (HIVAN). Two HIVd1443 transgenes were associated with the development of HIVAN (wild-type T26 and ΔNef X5 lines). In contrast, two ΔVpr transgenes (in V13 and VN15 mice) failed to induce HIVAN. A novel line bearing functional tat and vpr
genes (HIV[Tat/Vpr] mice) developed focal glomerulosclerosis (FGS) further implicating Vpr in HIV-1 provirus-induced FGS development. When vpr alone was transcribed from the murine c-fms promoter in transgenic mice, FGS was the principal histopathological lesion associated with the development of renal insufficiency. Both the HIV-1 LTR and the murine c-fms promoter are transcriptionally active in murine macrophages. Thus, in addition to demonstrating the ability of Vpr to induce FGS, the common features of the two model systems suggest that HIVAN modeled in transgenic mice is related to viral gene expression in macrophages, or macrophage-like cells.

The full extent of renal disease in wild-type HIVd1443 transgenic mice may require viral genes other than vpr. A transgene containing only the vpr gene (c-fms linked) is sufficient to induce the fundamental glomerulopathic lesion, but there is reason to suspect that tat and nef gene products contribute to HIVAN. Renal disease, both in terms of FGS and tubular degeneration, was enhanced in dual transgenic progeny of TV2 by HIVd1443Δ[Vpr] crosses compared to TV2 animals alone. In contrast, the complementation of HIVd1443Δ[VprΔNef] transgenic mice with vpr (by TV2 crosses) did not exacerbate disease beyond that characterizing TV2 mice. These observations implicate HIV-1 Nef in disease progression. Earlier Nef-complementation experiments (Kajiyama et al., 2000), and the incidence of interstitial inflammation in CD4/HIV transgenic models (Hanna et al., 1998), are consistent with this interpretation. Tat may have contributed directly to FGS in HIV[Tat/Vpr] mice because these animals were more compromised than c-fms/vpr transgenic animals. Alternatively, any Tat effect could have been indirect. The expression of vpr was higher in the kidneys of TV2 mice than in c-fms/vpr mice, perhaps because Tat transactivation of the viral LTR can occur in murine cells (Murphy et al., 1993). Likewise, posttranscriptional phenomena may explain the reduced expression of vpr in HIVd1443Δ[Nef] mice (relative to wild-type T26 mice) suggestive of an indirect effect of Nef. Importantly, the presence or absence of secondary HIV genes may have influenced spacing patterns, altering viral transgene expression in HIV transgenic mice (Fig. 5) in ways unrelated to transgene position effects. Whether viral gene products such as Tat and Nef contribute directly to renal disease remains a matter requiring clarification.

Pathologic changes in human HIVAN occur in the glomerular epithelia, notably podocytes, and the central lesion in HIVAN is focal glomerulosclerosis (FGS) (D’Agati and Appel, 1998). A direct effect of HIV-1 on glomerular podocyte proliferation has been proposed both for HIV-1-infected human podocytes and podocytes in HIVd1443 transgenic animals (Barisoni et al., 1999; Nelson et al., 2002). The development of FGS in c-fms/vpr transgenic animals strongly suggests that glomerular macrophages play a role in HIVAN. In exon 2 c-fms/EGFP transgenic mice, c-fms promoter activity was restricted to macrophages in the renal epithelia (Sasmono et al., 2003). There is evidence however that human and murine mesangial cells express c-fms (Mori et al., 1990; Watanabe et al., 2001). If mesangial cell expression of c-fms is much less than that in macrophages, it is possible that c-fms expression in mesangia of c-fms/EGFP transgenic mice (Sasmono et al., 2003) escaped detection. Anomalies have been attributed to murine mesangial cells in HIVd1443 mice (Singhal et al., 1998), thus mesangium remain a potential source and target of toxic viral gene products in humans as well as our murine models. Whatever the cell type expressing Vpr (glomerular epithelia or macrophages), pathologic effects could be exerted on proximal bystander cells. This could be due to the action of extracellular Vpr (Azad, 2000) or alterations in the local production of host cell factors (cytokines, growth factors). It seems clear however that endogenous renal expression of HIV-1 products is necessary for glomerulopathy to develop (Bruggeman et al., 1997).

Viral gene expression in kidneys of HIVd1443 transgenic mice was first observed just before weaning age (Dickie et al., 1991). The factors inducing viral gene expression at this time are unknown. Considering the transient nature of proteinuria in HIVd1443 mice, and the edema peculiar to nursing transgenic mice, it is reasonable to invoke a role for host factors in disease development. The enhanced morbidity in nursing mice may be related not so much to increased Vpr expression, but to the sensitivity of glucocorticoid responsive genes to modulation by Vpr (Kino et al., 1999). One possibility is that Vpr expressed in a critical subset of kidney cells induces renal damage by augmenting glucocorticoid responsiveness. This hypothesis is credible given the renal toxicity of dexamethasone (Chen et al., 1998) and the capacity of glucocorticoids to modulate the turnover of extracellular matrix by glomerular mesangium (Eberhardt et al., 2002; Kuroda et al., 2002). In view of its well-documented effects on cell-cycle control (Trono, 1995), Vpr toxicity may prove to be multifactorial.

The HIV LTR is responsive to glucocorticoid action and inflammatory stimuli. Were Vpr to enhance glucocorticoid action in the context of glomerular disease, an augmenting effect on HIV gene expression might have been observed. This interplay may explain, in part, why HIV-linked transgenes were associated with higher levels of vpr expression than was the c-fms-linked transgene. The exon-2 c-fms promoter contains no glucocorticoid response element (Sasmono et al., 2003). The combined effects of Vpr action and disease development could elevate HIV LTR-linked expression to levels not attained by the c-fms/vpr transgene.

In addition to clarifying the involvement of HIV-1 gene products in HIVAN, HIV transgenic model systems should be useful in understanding and targeting infected macrophages in counteracting HIV-1 persistence. It is important in the application of sustaining antiretroviral therapy to understand the concerted impact of viral gene products on proviral activity and immune dysfunction in tissue macro-
phages. Illuminating the unique characteristics of HIV-positive macrophages should lead to the conception of novel approaches to augment normal cellular immune responses and, better, to effect the removal of persistently infected cells from HIV-infected individuals.

Materials and methods

Transgene construction and animals

To create ΔGag/Pol proviral HIV transgenic mouse lines deficient in Vpr, or Vpr and Nef, frameshift mutations were introduced into pEVD1443 HIV DNA (Felser et al., 1989) and pEVD1443[ΔNef] DNA (Kajiyama et al., 2000) by cleavage at an EcoRI restriction enzyme site in the vpr gene (Fig. 1). DNA ends were blunt-ended with DNA polymerase I and rejoined with DNA ligase. In preparation of pTV2, the source of HIV[Tat/Vpr] transgene DNA, nef was deleted from pEVD1443 by excising the sequence between the unique BamHI site (position 8465) and KpnI at position 9005 and replacing it with the vpr open reading frame between positions 5480 and 5895 (Rsal sites) by blunt-end ligation. The sequence between NsiI and SspI sites of this plasmid (base 1247 to base 6153) was removed and replaced with a 395 bp (5348–5743) fragment produced by PCR amplification of the vpr gene (digested with PstI and EcoRI) and a 410 bp EcoRI/SspI fragment (5347–6153) by three-fragment ligation. The end-product, plasmid pTV2, contained a proviral derivative in which vpr genes flanked tat in its native position. MeRV mice carried a c-fms/vpr transgene derived from p7.2fms-EGFP (Sasmono et al., 2003) in which the Rsal vpr fragment replaced EGFP. For embryo microinjection, plasmid DNAs were digested with FspI and Nael (HIV), or PvuI (c-fns/vpr), and purified from agarose. Microinjection of fertilized FVB/N × FVB/N embryos followed standard procedures (Hogant et al., 1986).

FVB/N mice were purchased from Taconic Farms Inc. (Germantown, NY). Transgenic mouse lines were maintained in the hemizygous state through backcrosses with FVB/N mice. Transgenic offspring were identified either by PCR analysis of mouse genomic DNA. They were培育 in the hemizygous state through backcrosses with FVB/N mice. Transgenic offspring were identified either by PCR analysis of mouse genomic DNA. They were

Urine analyses

Spontaneously released urine samples were collected and analyzed immediately for protein and creatinine content. Protein (mg/dl) was determined using Bradford reagent (Bio-Rad Lab., Hercules, CA). Urine creatinine levels were measured using a commercially available kit (Sigma Corp., St. Louis, MO). Individual animals were tested on three successive days and mean values computed.

Histochemistry and electron microscopy

For histochemical analysis, kidneys from 1- to 2-month-old female mice were halved by a longitudinal midline cut and fixed in 10% buffered formalin. Five-micrometer sections were stained with hematoxylin and eosin. A pathologist (RU) blinded to the animal genotype performed the histological evaluation of c-fms/vpr transgenic tissue. Frozen sections (4 μm) of kidneys (in OCT) were immunostained with rabbit polyclonal antiserum raised against a peptide of Vpr (residues 1–46; AIDS Reagent and Reference Depository, Rockville, MD). After washing, the sections were incubated with Alex-488 conjugated goat anti-rabbit IgG (Molecular Probes, Oregon). For electron microscopy, renal tissue from 25-week-old mice was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer and embedded in glycolmethacrylate. Thin sections were stained with osmium tetroxide and examined by transmission electron microscopy.

Northern blot analyses

Total RNA (40 μg for kidney, 20 μg for other tissues), extracted using an acid-guanidinium procedure (Chomczynski and Sacchi, 1987), was fractionated in 1.25% denaturing (2.2 M formaldehyde) agarose and blotted onto Nytran membranes (Schleicher and Schuell Inc., Keene, NH). UV cross-linked membranes (Stratagene, La Jolla, CA) were hybridized with a 32P-dCTP-labeled probe derived from a Nef cDNA for the detection of HIV-specific transcripts (Dickie et al., 1991). An RNA molecular weight ladder was obtained from Life Technologies (Bethesda, MD). Membranes were stripped and rehybridized with a 32P-dCTP-labeled probe for human β-actin (Clontech, Palo Alto, CA) to control for loading discrepancies. The animals represented were between 24 and 35 days old.

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<tr>
<th>Table 2</th>
<th>PCR primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Primer sequence (5’–3’)</td>
</tr>
<tr>
<td>PNDN</td>
<td>GGTTGGGAGCAGTATGAGAATTGACCT</td>
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<tr>
<td>PNDN2</td>
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<tr>
<td>LVI1</td>
<td>GAGGACAGATGGAACAAGCC</td>
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<tr>
<td>RV1</td>
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<tr>
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<tr>
<td>VH2</td>
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<td>045</td>
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<td>R3</td>
<td>TACAGATCTACGCTGTCCT</td>
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<td>5FMS</td>
<td>CCCAGGGCTACGTCCCCATCTTTTCTCTC</td>
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<td>5GAPDH</td>
<td>AATGCATCTCGCAACACACAA</td>
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<tr>
<td>3GAPDH</td>
<td>GATACGTATCCGACGCATTTGATGCT</td>
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</table>

The primers used for PCR genotyping of HIV-1 transgenic mice and for RT-PCR analysis of tissue RNA are listed. HIV-specific sequences and base positions were derived from the sequence of HIV-1 proviral DNA in pEVD1443 (Felser et al., 1989). Asterisk (*) denotes base position of primer in p7.2fms-EGFP.
Transgenes were routinely detected in mouse genomic DNA by PCR using the primer pairs listed in Table 2. For the detection of HIVd1443 sequences: PDN3/PDN2; for TV2 sequences: LV1/PDN2; and for c-fms/vpr sequences: 5FMS/RV1. For RT-PCR detection of native vpr transcripts, the primer pair L2/VH2 was used with HIV transgenic lysates and LV1/RV1 was used for c-fms/vpr transgenic lysates. Alternative spliced products in HIVd1443 lysates were analyzed using the 045/RV1 primer pair. The 3′ vpr gene transcript of the TV2 transgene was detected with LV1/R3. GAPDH primers are also listed in Table 2. Semiquantitative RT-PCR was performed on total RNA or mRNA partially purified by a single selection on oligo-dT cellulose. Quantitative RT-PCR was performed on total RNA or mRNA amplification in triplicate. PCR cycles were determined by two- and tenfold serial dilutions of cDNA were subjected to PCR with Superscript II (Life Technologies). Undiluted, two- and fivefold serial dilutions of cDNA were subjected to PCR amplification in triplicate. PCR cycles were determined empirically to yield linear results. Standard amplification conditions were 94 °C for 2 min, 60 °C for 1 min, 75 °C for 1.5 min, 94 °C for 30 s. For the labeling of PCR products, 32P-dCTP (0.13 μM) replaced cold dCTP in the reaction mix. Unlabeled products were visualized in 1% agarose gels stained with ethidium bromide. Labeled products were fractionated in 5% acrylamide and exposed to X-ray film. The DNA ladder was obtained from Life Technologies.

Computational analyses

Individual values were computed as the average of triplicate experiments. Group means were subjected to statistical analyses (t test) using SigmaPlot8.0 graphics software (SSPS Science). Error bars denote single standard deviations.

Acknowledgments

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References


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