Viral Protein R of Human Immunodeficiency Virus Types 1 and 2 Is Disposable for Replication and Cytopathogenicity in Lymphoid Cells

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Viral protein R (VPR) is conserved in human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2). To assess its function, we have constructed mutations within the vpr coding regions of HIV-1 and HIV-2 predicted to express truncated VPR products. Influenza virus was produced by each proviral clone and showed similar replication kinetics and cytopathogenicity when compared with the corresponding parental proviral clone.

The human immunodeficiency virus type 1 (HIV-1) genome includes three genes, gag, pol, and env, encoding virion proteins, and six genes encoding proteins which potentially regulate virus replication (6, 13). The regulatory proteins include transactivator proteins: transactivator regulator of virion and regulatory proteins; virion and cytopathic effects includes three amino acids. In HIV-1, HIV-2 encodes VPX, which is needed for infection of certain cell strains, and HIV-1, HIV-2 encodes VPR, whose function is dispensable for replication and 5204 and 5205, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids.

The vpr gene is found in the central portion of lentivirus genomes. In HIV-1 it overlaps vif at its 5’ end, and in some HIV-1 strains it overlaps tat at its 3’ end (16). In different HIV-1 strains, the predicted protein products are 78 to 96 amino acids long and vary by 0 to 7% of amino acids within the first 78 amino acids. Thirty-four percent of HIV-1-infected individuals and some uninfected individuals possess antibodies reactive with a recombinant VPR product. This provides evidence for expression of the gene product in vivo.

In HIV-2, vpr follows vif and vpx and precedes tat; it is predicted to encode a protein of 105 amino acids (5). The VPR products of HIV-1 and HIV-2 are well conserved, with 52% amino acid homology, only slightly less than that of GAG and POL products (16). The vpr gene is also found in simian immunodeficiency virus from rhesus macaques (1) but not from African green monkeys (4). A similar sequence is also found in the visna virus genome (15).

To analyze the function of the HIV-1 VPR protein, we constructed three oligonucleotide-directed mutations (15) by using a functional proviral clone, pX (2, 12). Oligonucleotides were synthesized and used for mutagenesis (9) to create termination codons at positions 3, 23, and 32 of the vpr gene product. The mutation at codon 3 was created by a cytosine-to-thymidine transition at nucleotide position 5145 of the parental genome, resulting in a 2-amino-acid VPR product. At codon 23, a termination codon and a unique XbaI site were created by inserting a cytosine residue between positions 5204 and 5205, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids. At codon 32, a termination codon and a unique XbaI site were created by the insertion of a cytosine residue between positions 5203 and 5204, resulting in a predicted VPR product of 22 amino acids.

In a mutation in a functional HIV-2 proviral clone (pSE) (10; our unpublished results) was constructed by a guanosine-to-thymidine transversion at position 5700, thus introducing a termination codon at VPR residue 7 and eliminating a Sacl restriction enzyme site. This clone is designated pMR7. An additional clone, designated pMRR, predicted to encode a full-length VPR product, was constructed with a change of an adenosine to a cytosine at position 5738, introducing a SacI restriction site.

Virus production from each HIV-1 proviral clone was assessed by transfection of COS-1 or Jurkat cells and measurement of soluble p24 antigen production by enzyme-linked immunosorbent assay (Du Pont Co.). No significant differences in virus production were detected in cells transfected with pX compared with those transfected with pR2, pR22, pR31, or pR40 (results not shown). Furthermore, no differences were noted in TAT production by these clones as measured by their ability to trans-activate HIV-1 long terminal repeat-directed gene expression (results not shown).

To produce virus stocks from each proviral clone, we cocultivated H9 or CEM lymphoid cells with transfected COS-1 cells. Total cellular DNA was isolated from the infected cells and analyzed by Southern blot hybridization (Fig. 1). These data demonstrate that there are equivalent levels of proviral DNA in each infected cell line, as shown by the similar intensity of the hybridization signals (Fig. 1a, lanes 1 to 4; Fig. 1b, lanes 1 and 2; Fig. 1c, lanes 1 and 2; Fig. 1d, lanes 4 and 5; Fig. 1e, lanes 2 and 3). The presence of the mutation in R22 (Fig. 1a) and R31 (results not shown) was confirmed by the demonstration of the presence of an XbaI site, as indicated by the presence of a 2.8-kilobase (kb) DNA fragment after digestion with BamHI and XbaI (Fig. 1a, lane

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FIG. 1. Analysis of DNA sequences in HIV-1- and HIV-2-infected cells. Total cellular DNA was isolated from H9 cells infected with X, R2, R22, or R40 (a to c) or CEM cells infected with SE. MRR, or MR7 (d and e). Each DNA sample (10 μg) was digested with either XbaI and BamHI (a), EcoRI (b), NcoI and BglII (c), BglII and SacI (d), or BglII and SmaI (e). Controls include DNA from uninfected H9 or CEM cells or the same DNA to which 0.1 ng of cloned plasmid DNA from each proviral clone was added. Blots were hybridized with hexamer primer-labeled probes that include nucleotides 222 to 9213 of HIV-1 (a to c) or nucleotides 503 to 8598 of HIV-2 (d and e). Numbers to the left of each panel represent the size of the DNA fragments in kilobases.

2). The mutation in R40 was shown by the loss of the Neol site (Fig. 1c, lane 2). The mutation in MRR was confirmed by the presence of a SmaI site demonstrated by the cleavage of the 8.1-kb DNA fragment to 5.2- and 2.8-kb fragments with BglII and SmaI (Fig. 1c, lane 3). The mutation in MR7 was confirmed by demonstrating the loss of a SacI site in the polymerase chain amplified product of DNA from the infected cells (results not shown). These data suggest that fewer than 10% of the viral genomes have reverted.

Results of Southern blot analysis of restriction enzyme digests of the recombinant proviral DNA clones are also presented for comparison (Fig. 1a, lanes 5 to 8; Fig. 1b, lanes 3 and 4; Fig. 1c, lanes 4 and 5; Fig. 1d, lanes 2 and 3; Fig. 1e, lane 1). It should be noted that EcoRI digestion of plasmids pR2 and pX generates hybridizing bands of 16.0 and 1.1 kb, whereas the EcoRI digestion products of infected-cell DNA are about 9.0 and 1.1 kb, owing to the loss of plasmid DNA sequences (Fig. 1b).

FIG. 2. Viruses from HIV-1 VPR mutant clones demonstrate similar infectivity, replication, and cytopathogenicity to virus from the parental clone. Equivalent amounts of X, R2, R22, R31, and R40, as determined by reverse transcriptase and soluble p24 antigen measurements, were used to infect Molt-3 cells by using an undiluted, 5-fold-diluted, or 25-fold-diluted virus stock. (a) Reverse transcriptase activity was measured with eightfold-concentrated samples of conditioned media at the indicated time points. (b) The number of cells in each culture infected with undiluted virus stocks was determined with a hemacytometer.
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HIV-1 or HIV-2
replication,
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Although
the
VPR
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synthesized in infected individuals (16),
currently available serums have not detected VPR in
infected
cells.
It
is
possible that VPR has subtle regulatory
effects on the virus life cycle, which may not have been
detected in the current study. Furthermore, although a broad
range of cell lines and normal peripheral blood lymphocytes
were examined in this study, effects of VPR in other cell
types cannot be excluded. Another possibility is that the vpr
genes from the particular HIV-1 or HIV-2 clones used in this
study were partially or completely inactive. Alternatively,
VPR may regulate virus replication in vivo in ways that are
not detectable in our tissue culture models.

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