Genetic and functional characterization of human immunodeficiency virus type 1 VprC variants from north India: presence of unique recombinants with mosaic genomes from B, C and D subtypes within the open reading frame of Vpr

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The human immunodeficiency virus type 1 (HIV-1) epidemic in India is predominantly caused by genetic subtype C, though other minor subtypes have also been reported. One of the major accessory proteins of HIV-1, namely Vpr, is known to influence key steps in viral replication, cell cycle progression, promoter activation, apoptosis and pathogenesis. Therefore, we carried out a genetic and functional analysis of the Vpr variants from eight HIV-1-infected individuals from north India. The sequence analyses revealed that six of eight samples clustered with ancestral subtype C. Remarkably, five of these showed a conserved and region-specific L64P mutation, located in the predicted third α-helix. This change adversely affected their ability to activate the HIV-1 long terminal repeat promoter without compromising their ability to cause apoptosis. Bootscan, phylogenetic and SimPlot analysis of the remaining two samples (VprS2 and A6) revealed very interesting mosaic genomes derived from B, C and D subtypes. The N-terminal half of the VprS2 gene consisted of genomic segments derived from subtypes B/D, C and D but the C-terminal half was derived predominantly from subtype C. Interestingly the N-terminal half of sample A6 also showed similar B/D, C and D inter-subtype recombinant structure but the C-terminal half was entirely derived from the consensus B subtype. Multiple breakpoints in a short stretch of 291 nt encoding the Vpr gene strongly suggest that this region is a potential hot-spot for the formation of inter-subtype recombinants and also highlight the importance of the rapidly evolving HIV-1 epidemic in the north Indian region due to multiple genetic subtypes.

INTRODUCTION

The human immunodeficiency virus type 1 (HIV-1) AIDS epidemic continues to spread at a rate of 13 000–14 000 new infections per day. According to the UNAIDS estimate, there are approximately 33 million HIV-1-infected individuals and approximately 25 million have already died. HIV-1 isolates from all over the world have been divided into three groups, namely M, N and O. The M group of viruses consists of at least nine pure subtypes and numerous circulating recombinant forms and unique recombinant forms (Robertson et al., 1999). Although different genetic subtypes have been reported from all over the world, more than 50 % of all HIV-1 infections are due to genetic subtype C, which is predominant in South Africa, India and China (Cassol et al., 1996; Dietrich et al., 1993; Galai et al., 1997; Hu et al., 1996; European Commission & UNAIDS, 1997). This is in sharp contrast with the USA and the UK, where subtype B is the predominant subtype. HIV-1 infection mainly spreads through heterosexual transmission. High rates of infection (>3 million each year) are reported from sub-Saharan Africa and account for approximately 65 % of new infections. Although the HIV-1 AIDS epidemic occurred late in Asia, it is now recognized as the second most explosive epidemic, with more than 1 million infections annually. In India alone, there are more than 3 million
HIV-1-infected individuals, predominantly due to subtype C, offering a critical target for vaccine design. Genetic analysis or molecular epidemiology has been a vital tool for understanding the origin of various genetic subtypes and to monitor their global spread. Lately, new recombinants have been reported from various regions due to the presence of co-circulation of more than one subtype. These new recombinants may play a major role in shaping the epidemic (Carr et al., 1998; Takebe et al., 2008). It is obvious that global molecular epidemiology has immense biological and public health implications, especially in designing region-based vaccines. HIV-1 Vpr plays an important role during virus replication, reverse transcription, nuclear import, cell cycle progression, the ability to cause apoptosis and also in its ability to activate the HIV-1 long terminal repeat (LTR) promoter and other cellular promoters (Le Rouzic & Benichou 2005). Recent NMR-based structural studies suggest that the Vpr protein consists of three helical domains and three turns. It contains multiple leucine-rich domains important for its function (Moret et al., 2003; Thotala et al., 2004). The purpose of carrying out this study was to find out the level of genetic change that is present in the Vpr gene from HIV-1-infected individuals from north India (Punjab/Haryana) and to find out its possible functional relevance. We recently reported subtype B- and C-specific differences in Vpr-mediated cell cycle progression (Bano et al., 2007a) and in their ability to activate the HIV-1 LTR promoter and cause apoptosis (Bano et al., 2007b). Although genetic variations among several HIV-1 genes such as nef, env and ltr have been reported from different regions of India (Jere et al., 2004; Rodriguez et al., 2007; Bhanja et al., 2007), we provide data on the genetic and functional analysis of Vpr genes from the Punjab/Haryana region of north India, which show the involvement of other subtypes in infections in this region.

METHODS

Patient population. Eight unrelated, HIV-1-infected individuals (S1–S3 and A4–A8) from the Punjab/Haryana region of north India (immediately north of the Indian capital, Delhi) were selected on a random basis for our studies. They were studied at the Immunodeficiency Clinic of the Post Graduate Institute of Medical Research and Education, Chandigarh, after obtaining all the required clearances. The clinical history of each individual is given in Table 1. The CD4 and CD8 cell count was enumerated by a bead-based FACS count kit (BD Biosciences Immunocytochemistry System). Peripheral blood mononuclear cells were collected from peripheral blood and genomic DNA was isolated as described previously (Husain et al., 1998).

Genomic DNA isolation and amplification of the Vpr gene. Genomic DNA was isolated using a genomic DNA extraction kit (Qiagen). The sequence spanning the Vpr gene was amplified by PCR. All PCR primers were performed with high fidelity Taq polymerase. The following primers were used: (i) forward, BamHI, 5’-gcGGATCC-ATGGGAAAGGCCCCAGAAG and (ii) reverse, XhoI, 5’-gcCTCGAG-CATTGGATCTACTGGCTCCATT TC. For cellular studies, BamHI and XhoI restriction sites (underlined above) were engineered at the ends of forward and reverse primers, respectively, for subcloning into pCDNA 3.1 expression vector (Invitrogen) under the cytomegalovirus (CMV) promoter. The gel-purified PCR products were cloned initially in pGEM-T Easy Vector (Promega) and subsequently in pCDNA 3.1 (referred to as Vpr constructs). Using the same primers, we also amplified the full-length Vpr genes from pNL4-3 (Adachi et al., 1986) and 93IN905 Indian genetic subtype C (Loel et al., 1999) DNA. These were used as controls for all our cellular studies.

Sequencing, computer alignment and analysis of the Vpr gene. Forward and reverse sequencing of the Vpr variant genes were performed with T7 and SP6 universal primers. Sequence alignment was carried out in CLUSTAL W software (http://www.ebi.ac.uk/Tools/clustalw2/index.html), edited manually and compared with consensus B and C sequences (Fig. 1). The bootstrap analysis was performed in SimPlot version 3.5.1 using Kimura two-parameter (Salminen et al., 1995). The radial phylogenetic trees were constructed using the neighbour-joining method with Kimura distance and visualized inTreeView (Win16) version 1.4.0. The consensus and reference sequences were retrieved from an HIV database located in Los Alamos (http://www.hiv.lanl.gov). A mountain plot graph of the predicted RNA structure was constructed using the methods described earlier (Galli et al., 2008).

Determination of dS/dN ratio. The proportion of non-synonymous substitutions (dS) per potential synonymous site and the proportion of synonymous substitutions (dN) per potential non-synonymous site were calculated using the program SNAP (http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html) developed by Nei & Gojobori (1986) and Ota & Nei (1994).

Cell line. 293 T cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) with 10 % fetal bovine serum (Biological Industries).

HIV-1 LTR promoter activation with Vpr protein variants. 293 T cells were transfected with 0.1 μg HIV-1 LTR luciferase reporter construct ml−1 along with the various Vpr constructs (1 μg each). Twenty-four hours post-transfection, cell lystate was prepared using reporter lysis buffer (Promega) and luciferase activity was measured by a luminometer as described previously (Bano et al., 2007b). To normalize transfection efficiency, a reporter gene encoding vector (pSV-β-gal) was used with each transfection. The data were analysed using nonparametric methodology (Mann–Whitney U test) where P<0.05 was considered to be significant.

Apoptosis. 293 T cells were transfected with Lipofectamine (Invitrogen) for 48 h with the plasmids encoding wild-type VprB,

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age (years)</th>
<th>Sex</th>
<th>CD4 count</th>
<th>CD8 count</th>
</tr>
</thead>
<tbody>
<tr>
<td>NII-PGI-IND-S1</td>
<td>33</td>
<td>M</td>
<td>364</td>
<td>1543</td>
</tr>
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<td>M</td>
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<tr>
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<td>F</td>
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<tr>
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<td>1064</td>
</tr>
<tr>
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<td>M</td>
<td>519</td>
<td>2000</td>
</tr>
<tr>
<td>NII-PGI-IND-A8</td>
<td>39</td>
<td>M</td>
<td>447</td>
<td>1346</td>
</tr>
</tbody>
</table>

ND, Not determined. All individuals contracted HIV-1 via heterosexual transmission.
Fig. 1. (a) Sequence analysis of Vpr variants. The NII-PGI-IND-Vpr (S1–S3 and A4–A8) amino acid sequences derived from our isolates were aligned and compared with the consensus C and consensus B sequences. Dots (.) indicate a match with consensus C; and asterisks (*) and double dots (:) indicate that, in the samples used in this study, the sequence is conserved and varied, respectively. Three predicted helical domains (1–3HD) are shown at the top of the sequences. The positions of the mutated amino acids are indicated above the consensus C sequence. (b) Phylogenetic analysis of Vpr variants. Six of our samples (all of which had the L64P mutation except sample A5) formed a cluster close to ancestral C. The two recombinant Vpr variants (S2 and A6) showed different branching close to consensus B and D.
VprC and the three Vpr variants (S1–S3). Thereafter, the cells were stained with propidium iodide (10 \( \mu \)g ml\(^{-1} \)) and analysed by using flow cytometry to determine the extent of apoptosis. FACS data were analysed using WINMDI version 2.9 software.

**Western blot analysis.** 293 T cells were grown to 80% confluency in a six-well plate and transfection of various Vpr constructs (1 \( \mu \)g each) was carried out with Lipofectamine. After 48 h transfection, the cells were harvested and incubated in cell lysis buffer (PBS containing 0.1% Triton X-100) and subjected to 12% PAGE. Equal amounts of protein were transferred to a nitrocellulose membrane (BIORAD). Polyclonal Vpr rabbit antiserum (obtained from the NIH) was used as the primary antibody and horseradish peroxide-conjugated rabbit IgG (Santa Cruz) was used as the secondary antibody. The blot was developed using ECL reagent (Amersham).

**RESULTS**

**Baseline characteristics of the population**

Table 1 summarizes the clinical characteristics of eight HIV-1-infected individuals from the Punjab/Haryana area of northern India. The subjects have all been seropositive for 5 years or more. Their CD4 counts varied from 250 to more than 1000 but their CD8 counts ranged from 1064 to 2000 at the time of blood collection (Table 1). All of the patients (except S1) had been undertaking standard first line antiretroviral treatment for more than a year.

**Sequence analysis**

Amino acid sequences from all eight samples were aligned and compared with consensus C and B subtype sequences (Fig. 1a). The locations of the three predicted \( \alpha \)-helix domains (1HD to 3HD) are shown at the top of the sequence. Most of the NII-PGI-IND sequences contained conserved changes in the second and third helical domains. One of the most conserved and remarkable features was the substitution of leucine-64 with proline (L64P) in the third helical domain of five of eight samples. Additionally, samples S1 and A5 showed R32S and W38R changes, respectively. The remaining two samples, S2 and A6, were unique recombinants (see detailed description below) with multiple substitutions that were common with either consensus B or consensus C. It must be noted that we characterized at least three or four separate clones derived from all the samples from each infected individual to ensure that they represented true mutations.

**Phylogenetic analysis of Vpr variants**

Phylogenetic analysis of all the NII-PGI-IND-Vpr samples showed that six (S1, S3, A4, A7, A8 and A5) of them formed a separate cluster that segregated with ancestral/consensus subtype C; interestingly, all of these samples except A5 possessed the L64P mutation. One of the two recombinant samples, VprS2, clustered with subtypes B and C but the A6 sample showed similarity to consensus subtypes B and D (Fig. 1b).

Genetic characterization of the Vpr variants

In order to characterize the nature of recombinants in more detail, the two recombinant samples (S2 and A6) were subjected to extensive bootscan and phylogenetic analysis using SimPlot version 3.5.1. Analysis of the S2 variant is shown in Supplementary Fig. S1(a) (available in JGV Online). This also shows the corresponding phylogenetic analysis of the same variant. The N-terminal half (nt 1–135) of isolate S2 consisted of B/D, C and D subtype sequences and the C-terminal half consisted of predominantly C. The N-terminal half (nt 1–132) of sample A6 also formed a mosaic structure consisting of B/D, C and D but the C-terminal half was derived from ancestral B (Supplementary Fig. S1b). We carried out a similar type of genetic analysis with a VprS1 variant that harboured the L64P mutation (Supplementary Fig. S1c) along with VprA5, which lacks this mutation (Supplementary Fig. S1d). These samples, as expected, displayed almost 100% similarity to ancestral subtype C when subjected to bootscan analysis.

**Breakpoint and hairpin formation analysis**

The two recombinants (VprS2 and A6) were further analysed for possible correlation between the location of breakpoints, entropy values and predicted hairpin structures by constructing a mountain plot as described by Galli et al. (2008). Both recombinants showed good correlation with distribution of breakpoints, lower entropy values and predicted hairpin structures (Supplementary Fig. S2). The N-terminal half showed more predicted hairpin structures than the C-terminal half.

**Rates of accumulation of non-synonymous and synonymous substitution**

The non-synonymous/synonymous ratio of rate constants \( (dS/ddS) \) is indicative of the selection pressure at the protein level. A \( dS/ddS \) ratio <1 is indicative of purifying selection and amino acid conservation because of structural and functional constraints; a \( dS/ddS \) ratio >1 is indicative of diversifying and positive selection where amino acid substitutions confer an advantage (Ganeshan et al., 1997). All eight Vpr variants (including the two recombinant clones S2 and A6) exhibited evidence of purifying selection as their \( dS/ddS \) ratio was uniformly <1 (Table 2).

**HIV-1 LTR promoter activation is significantly less in Vpr samples with an L64P mutation**

Next, we wanted to find out how these amino acid changes impacted on the two well-known functions of the Vpr protein, namely, HIV-1 LTR promoter activation and apoptosis, as described previously (Bano et al., 2007b). We selected three samples for this purpose: two were non-recombinant subtype C Vpr genes (S1 and S3), both
Table 2. Rates of accumulation of non-synonymous and synonymous substitution. The $d_{s}/d_{S}$ ratio indicates purifying selection

The $d_{s}/d_{S}$ ratio was determined for all eight Vpr variants using the SNAP program available at http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html, as described in Methods.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>$d_{s}/d_{S}$ Ratio</th>
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<tbody>
<tr>
<td>Vpr S1</td>
<td>0.27</td>
</tr>
<tr>
<td>Vpr S2</td>
<td>0.19</td>
</tr>
<tr>
<td>Vpr S3</td>
<td>0.27</td>
</tr>
<tr>
<td>Vpr A4</td>
<td>0.28</td>
</tr>
<tr>
<td>Vpr A5</td>
<td>0.55</td>
</tr>
<tr>
<td>Vpr A6</td>
<td>0.15</td>
</tr>
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<td>Vpr A7</td>
<td>0.29</td>
</tr>
<tr>
<td>Vpr A8</td>
<td>0.29</td>
</tr>
</tbody>
</table>

containing the L64P mutation, and one was a B/C/D recombinant (VprS2). They were cloned into the pCDNA 3.1 expression vector downstream of a powerful CMV promoter. We co-transfected these Vpr constructs (1 µg ml⁻¹) along with varying amounts of HIV-1 LTR-B luciferase plasmid DNA (0.1 µg ml⁻¹), as described previously (Bano et al., 2007b), and luciferase activity (RLU) was measured (Table 3). Samples with proline in the third $\alpha$-helix (S1 and S3) exhibited significantly less activity with respect to their ability to transactivate HIV-1 LTR promoter when compared with wild-type VprB or VprC ($P<0.05$). On the other hand, the recombinant VprS2 transactivated the HIV-1 LTR-B promoter activity significantly. Very similar results were obtained with another recombinant sample, A6. Control VprB and C showed about 7- and 4.5-fold more activation than the LTR-B reporter gene alone (Table 3). We engineered an L64P mutation in the prototype VprC gene (derived from 93IN905 genetic clone) and observed a 3.6-fold reduction in LTR promoter activation. Also, when VprC was mutated from leucine to alanine at aa 22, a twofold increase in HIV-1 LTR promoter activity was seen (Table 3).

**HIV-1 Vpr variants possess the ability to cause apoptosis**

To determine the relative efficiency of the Vpr variants (S1, S2 and S3), an apoptosis assay was performed in 293 T cells as described previously (Bano et al., 2007b) (Fig. 2a). As reported earlier, Vpr derived from an Indian C isolate (93IN905) (Fig. 2a, iii) showed equivalent amounts of apoptosis to VprB (Fig. 2a, ii). All three samples (VprS1–S3, Fig. 2a, iv–vi, respectively) were capable of inducing apoptosis to varying extents, all of which were significantly more than that observed in untransfected cells (Fig. 2a, i). Sample A6 was also as competent as VprS2 at causing apoptosis (~32%, data not shown). When the L64P mutation was engineered into either the VprB or the VprC (derived from 93IN905) gene, about 15–20% more apoptosis was seen compared with wild-type VprB or C (Fig. 2b).

**HIV-1 Vpr variants show similar intracellular stability**

It may be argued that Vpr variants with the L64P mutation fail to transactivate the HIV-1 LTR promoter due to the short half life of the protein itself or inefficient intracellular synthesis compared with control VprB or VprC proteins. In order to rule out this possibility, we transfected the same three Vpr variants (VprS1–S3) into 293 T cells along with VprB and C controls. Forty-eight hours post-transfection, the cells were lysed. Using Western blot analysis, we observed no significant difference in the intracellular levels of Vpr protein (using ImageJ program) with any of the Vpr constructs; the same was true for the control actin protein (Fig. 3), which showed no significant changes in the corresponding lanes. Control cells did not show any Vpr-specific protein band. We conclude that the Vpr protein is synthesized by various constructs with similar efficiency and stability.

**DISCUSSION**

The genetic characterization of the Vpr genes from eight samples from northern India revealed several interesting features that support the dynamic and evolving nature of the HIV-1 epidemic in this region of India, which indicates the presence of several subtypes, namely, B, C and D. Although the majority of the samples (six of eight) showed phylogenetic relatedness with Indian subtype C reference...
Fig. 2. (a) Variant Vpr proteins are capable of inducing apoptosis. Approximately $1 \times 10^6$ 293 T cells were transfected with pCDNA3 vector alone (i) or 1 μg VprB (ii) or VprC (iii) (controls) or the three variant constructs (VprS1–S3; iv–vi, respectively) in the presence of Lipofectamine for 48 h. Following this, they were stained with propidium iodide and monitored for apoptosis by FACS/DNA laddering as described previously (Bano et al., 2007b). Representative results obtained from three independent experiments are shown. Percentage apoptosis recorded with each variant is shown at the upper right corner. (b) L64P mutant Vpr proteins are capable of inducing apoptosis. HEK 293 T cells were transfected with either 1 μg VprB- or VprC-encoding plasmid, or with their L64P mutant constructs, as indicated. The cells were stained with propidium iodide after 48 h incubation at 37 °C. Percentage apoptosis recorded with each variant is shown at the upper right corner of all plots.
strain (93IN905) (Lole et al., 1999) or ancestral C, two of them were novel recombinants and may have implications in the design of vaccines in India. The selective reduction of L64P mutants with respect to HIV-1 LTR promoter activity may contribute to varying HIV-1 gene expression and pathogenesis. It is important to mention that we repeatedly observed that HIV-1 LTR reporter gene activity was significantly less in variants that harboured the L64P mutation (VprS1 and S3) compared with VprS2 or A6 (recombinants). This L64P mutation was also present in the circulating virus of samples S1 and S3 (data not shown). This is not surprising because VprB variants that were created by in vitro mutagenesis previously exhibited varying levels of LTR promoter activation (Thotala et al., 2004). This L64P mutation is present almost in the middle of the predicted third a-helix which is likely to interfere with the helix formation and the functions associated with this region. More molecular functional studies are needed to explain this diminished activity. This reduced ability to activate the HIV-1 LTR promoter was not due to different amounts of Vpr protein being synthesized intracellularly in the mammalian cells, as determined by Western blot analysis. Vpr sequence evolution, as determined by studying dS/dD, suggested no positive selection but uniformly purifying selection. Additional samples taken at different time points over the course of infection are required to address this question. It is noteworthy that the VprB L64P mutation (created by mutagenesis and not a natural isolate) was previously shown to enhance the pro-apoptotic potential of subtypes B and D but not against the A/G recombinant (Jian & Zhao, 2003). Another conserved feature in all our isolates except sample A6 is the presence of 77Q, which was previously shown to be associated with long-term non-progression of HIV/AIDS and impaired induction of apoptosis (Lum et al., 2003). The high number of CD4 positive cells in sample A6 suggests that this individual may take longer time to come down with HIV/AIDS. The Vpr gene of this individual has several novel mutations (T55A, R77Q) and I1 to VT at positions 83 and 84) which may have contributed to long-term non-progression. The majority of the sampled individuals showed normal progression to HIV/AIDS disease (A. Wanchu, unpublished observations) and their Vpr proteins were capable of inducing apoptosis. This is not surprising because genetic changes in one genetic subtype do not always correlate with the phenotypic changes observed with other subtypes (Jian & Zhao, 2003; Lum et al., 2003). None of our samples showed impaired apoptotic potential when compared with prototypical Vpr B or C proteins. This is not surprising because the apoptotic peptide containing a motif from aa 71–96 was fully conserved in all our Vpr variants. This peptide is known to induce apoptosis via a direct effect on the mitochondrial permeability transition pore (Jacotot et al., 2000). Other mechanisms of Vpr-mediated apoptosis have also been proposed (Andersen et al., 2006) and may be modulated to varying extents by our variants; however, this was not explored in this study. The Vpr protein is known to carry out multiple functions. The fact that non-recombinant variants activate the HIV-1 LTR promoter poorly but retain the ability to cause apoptosis may be important for understanding genetic subtype-specific pathogenesis.

Such extensive mosaicism in the short 291 nt stretch encoding Vpr not only strongly suggests that this region is a potential hot spot for the formation of inter-subtype recombinants but also highlights the rapidly evolving HIV-1 epidemic in this region of India. Interestingly, the break points observed at the N-terminal half of the two recombinants were common (with the exception of one or two nucleotides) with the exception of the C-terminal half, which was either B or C. These extensive cross-over events in the N-terminal half of the gene are supported further by predicted hairpin structures and lower entropy (mountain plot) that are known to facilitate recombination (Galli et al., 2008). Our observation of two novel B/C/D Vpr recombinants out of the eight samples follows the global trend that shows that recombinants are being generated with increasing frequency and occasionally they may be responsible for shaping and sustaining the epidemic. We have carried out genetic analysis of HIV-1 LTR sequences from some of these samples and reported novel B/C recombinants (Neogi et al., 2008) from northern India; we have also observed evidence of recombination in other HIV-1 genes (vif, vpu and nef) (manuscript in preparation). These recombinants will impact on the

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**Fig. 3.** Vpr variants are synthesized intracellularly with equal efficiency and stability. Approximately 1×10⁶ 293 T cells were transfected with Vpr constructs (0.1 µg plasmid DNA ml⁻¹) using Lipofectamine; the total cell lysate was prepared 48 h post-transfection and split into two equal aliquots that were used to estimate the levels of Vpr protein and as a control protein (actin binding). The Western blot was developed as described in Methods and previously (Bano et al., 2007a).
choice of T-cell-epitope-based vaccines against HIV-1 for this region of India. Since the HIV-1 LTR-mediated transactivation with Tat protein is far more significant than the Vpr protein, the Vpr variants are not likely to affect the virus replication in a tissue-culture-based assay. Vpr protein is, however, known to play an important role in pathogenesis under in vivo conditions, which could be evaluated in an animal model.

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REFERENCES


Bano, A. S., Gupta, N., Sood, V. & Banerjea, A. C. (2007b). Vpr from HIV-1 subtypes B and C exhibit significant differences in their ability to transactivate LTR-mediated gene expression and also in their ability to promote apoptotic DNA ladder formation. AIDS 21, 1832–1834.


