Growth ability of human immunodeficiency virus type 1 auxiliary gene mutants in primary blood macrophage cultures

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A strain of human immunodeficiency virus type 1 that is strictly tropic for primary human blood cell cultures was constructed in vitro. Mutational studies on the vif, vpr, vpu and nef genes of this virus were performed to evaluate their biological functions in natural target cells. For this purpose, replication properties of mutant viruses in peripheral blood mononuclear cells (PBMCs) and macrophages (PBMPs) were determined. Three phenotypes with respect to virus replication were noticed: normal or mildly retarded growth (nef and vpr mutants), impaired growth (vpu mutant), and no growth (vif mutant). These results suggest that the Vif and Vpu proteins are more important than the Nef and Vpr proteins for virus replication in PBMCs and PBMPs.

Human immunodeficiency virus type 1 (HIV-1) carries several genes not found in other retroviruses. Extensive mutation analyses of the HIV-1 genome have revealed that the vif, vpr, vpu and nef genes are not critical for infection and replication in established cell lines (for reviews, see Cullen & Greene, 1990; Desrosiers, 1992). However the products of these non-essential genes certainly modulate virus replication in vitro (Cullen & Greene, 1990; Desrosiers, 1992) and, more importantly, in vivo (Kestler et al., 1991; Lang et al., 1993). It is well known that HIV-1 isolates exhibit a remarkable degree of biological variability with respect to host range, replication rate, etc. (Anand et al., 1987; Asjo et al., 1986; Cheng-Mayer et al., 1988, 1989; Evans et al., 1987; Tersmette et al., 1988, 1989; Von Briesen et al., 1987). Previous studies (Cheng-Mayer et al., 1988; Tersmette et al., 1989) have shown that the development of AIDS is correlated with the emergence of HIV-1 variants that replicate more efficiently in a wide variety of different human cells. Most mutation studies so far reported have been done by using 'virulent' viruses as the wild-type (wt). The present study was undertaken to determine whether mutations in the non-essential genes of an avirulent virus strain with a restricted host range can influence its replication in natural target cells. We demonstrate here that a mutation in the vif or vpu gene drastically alters the phenotype with respect to virus replication in these cells.

Fig. 1 shows full-length molecular clones used in this study. An infectious molecular clone designated pNF462 was constructed by inserting the EcoRI-XhoI fragment derived from HIV-1 isolate SF162 (Cheng-Mayer et al., 1989) into the corresponding region of pNL432 (Adachi et al., 1986). On transfection into SW480 cells (Adachi et al., 1986), pNF462 generated progeny virions comparable to those produced by pNL432, as judged by production of reverse transcriptase (RT) (Willey et al., 1988) in culture media (data not shown). Cell-free virus samples prepared from SW480 cells transfected with pNF462 or pNL432 were inoculated onto various CD4+ cell lines to determine their host range. As shown in Fig. 2, although NL432 did not grow in monocytic cell lines [U937 (Ralph et al., 1976), HL60 (Collins et al., 1977) and THP-1 (Tsuchiya et al., 1980)], it grew fairly well in a variety of lymphocytic cell lines [H9 (Mann et al., 1989), A3.01 (Folks et al., 1985), Molt4-8 (Kikukawa et al., 1986), M8166 (Shibata et al., 1991), MT-4 (Harada et al., 1985) and CEMx174 (Salter et al., 1985)]. In contrast, NF462 did not grow in any of the nine cell lines, in the manner of one of the parental viruses, SF162 (Cheng-Mayer et al., 1989). From the pNF462 clone, five frameshift mutant plasmids designated pNF-Nd (vif mutant), pNF-Ec (vpr mutant), pNF-Av (vpu mutant), pNF-Xh (nef mutant) and pNF-AE (vpr-vpu double mutant) were generated (Fig. 1) to evaluate their biological function. The double mutant pNF-AE, and another double mutant of plasmid pNL432 designated pNL-AS (Fig. 1) were constructed to test the hypothesis.
Short communication

Fig. 1. Structures of HIV-1 proviral clones used in this study. Construction and characterization of wt clone pNL432 and its derivative plasmid mutants designated pNL-Af and pNL-Ss have been described previously (Adachi et al., 1986; Ogawa et al., 1989). The vpr–vpu double mutant of plasmid pNL432, designated pNL-AS, was constructed by replacing the EcoRI–XhoI fragment of pNL-Af with the corresponding fragment of pNL-Ss. To generate another wt clone designated pNF462, the EcoRI–XhoI fragment derived from the SF162 isolate (Cheng-Mayer et al., 1989) was inserted into the EcoRI and XhoI sites of pNL432. Various frameshift mutant clones derived from pNF462 were constructed by cleavage of plasmid DNAs with the restriction enzymes indicated, blunt ending by T4 DNA polymerase, and rescaling by T4 DNA ligase. Plasmid pNF-Nd (vif mutant; out of 192 residues it encodes 28 amino acid residues from the N terminus identical to the wt sequence, i.e. 28/192) has a 2 bp insertion; pNF-Ec (vpr; 62/96) and pNF-Xh (nef; 37/208) have 4 bp insertions; pNF-Av (vTu; 24/81) has a 10 bp insertion; pNF-AE (vpr and vpu) has the mutations of both pNF-Ec and pNF-Av. Restriction sites used for making various clones are indicated. DNA structures of the mutant clones were determined by restriction mapping and partial sequencing to ensure that the mutations introduced do not affect other genes. LTR, Long terminal repeat.

(Westervelt et al., 1992) that the Vpu and Vpr proteins are capable of functional complementation in monocytes.

The growth potential of these mutant viruses was tested in human peripheral blood mononuclear cells (PBMCs). Fig. 3 shows representative results. Whereas the nef mutant grew with an efficiency similar to wt virus, replication of the vif mutant was not detected. The other three mutant viruses showed somewhat attenuated replication in PBMCs, particularly the vpu mutant NF-Av. Because the vpr–vpu double mutant NF-AE grew more slowly than the vpu mutant (and the vpr mutant), the mutation in the vpr gene appeared to have little effect on the growth rate of the virus. The growth rates of the mutants were then determined in peripheral blood macrophages (PBMPs) (Fig. 4). Two independent preparations of PBMPs were made, and used for infection. Again in these cells, the nef mutant replicated with similar kinetics to wt virus growth, and the vif mutant did not grow at all. Strikingly, the vpu mutant displayed a severe replication defect in the PBMPs. The mutation in the vpr gene resulted in a mild biological effect as judged by the growth kinetics of the vpr mutant and vpr–vpu double mutant.

The data presented in Fig. 3 and Fig. 4 indicated that in PBMCs and PBMPs the requirement for the Vpr protein for virus replication was less clear. However the mild effect on replication rate caused by mutation of the vpr gene was not confined to that observed in the primary cell cultures (Ogawa et al., 1989). As shown in Fig. 5, the effect caused by mutation of the vpu gene on virus replication was readily observed in cell lines such as A3.01 and H9. In contrast, the biological effect of vpr mutation on replication properties (NL-Af and NL-AS in Fig. 5) was not clear in A3.01 cells.

In this study, biological effects of mutations in non-essential genes on virus replication properties were examined. The parental wt virus clone NF462 (Fig. 1) used in this report did not productively infect any lymphocytic or monocytic cell lines tested (Fig. 2), and grew only in primary PBMCs and PBMPs (Fig. 3 and Fig. 4). Five mutant viruses derived from this typical attenuated strain, NF462, were evaluated, by RT assays, for their ability to grow in these natural target cells, and
a number of interesting findings were noted. Qualitatively and quantitatively similar results were obtained by antigen (Gag p24) capture assays (data not shown).

Among the four non-essential genes, \textit{vif} was the only ‘essential’ gene for virus replication in primary blood target cells as reported previously (Akari \textit{et al.}, 1992). In contrast, the \textit{nef} and \textit{vpr} genes, particularly the \textit{nef} gene, were not required for normal virus growth in these target cells. However it has been shown recently that the Nef and Vpr proteins are important for virus replication \textit{in vivo} (Kestler \textit{et al.}, 1991; Lang \textit{et al.}, 1993). Whether these two genes are necessary for virus replication in cells other than those examined here remains to be investigated. Interestingly, the \textit{vpu} gene was fairly critical for virus replication in PBMPs (Fig. 4). It has been suggested that the \textit{vpu} and \textit{vpr} genes may be capable of functional
Fig. 3. Growth kinetics of NF462 and mutant viruses in PBMCs prepared by the method of Ohta et al. (1988). Virus samples obtained as described in the legend to Fig. 2 were inoculated onto PBMCs (10⁶), and RT production in the culture fluids was monitored at intervals. Symbols: O, NF462 (wt); ●, NF-Ec (vpr mutant); □, NF-Xh (nef mutant); ■, NF-Nd (vif mutant); ▲, NF-Av (vpu mutant); △, NF-AE (vpu-vpr double mutant); ○, mock infection.

Fig. 4. Growth kinetics of NF462 and mutant viruses in PBMPs, which were prepared by the method of Gartner et al. (1986). They were trypsin-resistant, almost 100% positive for non-specific esterase (Sigma), and 75% MAC-1-positive (Daishinbo seiyo) when used for infection. PBMPs [confluent culture in one well of a 24-well plate (Corning)] were infected with 5 x 10⁵ RT units of cell-free virus samples as described in the legend to Fig. 2, and monitored for RT production at the designated intervals. Symbols as in Fig. 3.

Fig. 5. Growth kinetics of NL432 and mutant viruses in T cell lines. Methods for infection were as described in the legend to Fig. 2 except for input virus dose. Lymphocytic cell lines A3.01 and H9 (10⁶ cells) were infected with 5 x 10⁵ RT units. Symbols: ○, NL432 (wt); ●, NL-Af (vpr mutant); ▲, NL-Ss (vpu mutant); △, NL-AS (vpr-vpu double mutant); ○, mock infection.

complementation in primary monocytes (Westervelt et al., 1992). However from our data, there was no good reason to support this hypothesis. A longer delay in growth kinetics displayed by the vpu–vpr mutant virus relative to the kinetics of the vpu or the vpr mutant was generally observed in a T cell line (Fig. 5) and primary cells (Fig. 3 and Fig. 4). Mutations in virus genes that encode proteins acting independently might result in this phenotype.
This work was supported in part by a grant-in-aid for AIDS research from the Ministry of Education, Science and Culture of Japan. Meiko Kawamura holds a Research Resident Fellowship Award from the Japanese Foundation for AIDS Prevention.

References


