Growth characteristics of T-cell tropic HIV-1 *vpu* gene mutants in human peripheral blood mononuclear cells

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Abstract : A mutant designated NL-E65, which lacks the expression of entire *vpu* gene, was constructed from T-cell tropic wild-type (wt) human immunodeficiency virus type1 (HIV-1) clone and monitored for its replication property in human cells, along with a mutant NL-Ss which expresses a C-terminal truncated Vpu. The mutant NL-Ss could grow in two cell lines and in all peripheral blood mononuclear cell (PBMC) preparations to some extent, with kinetics similar to those of wt virus. Likewise, the mutant NL-E65 exhibited a replication property typical to the *vpu* mutant in the two cell lines and in all PBMC cultures, growing at a low level. Along with the results previously reported, these data indicate that HIV-1 Vpu is dispensable for virus replication in any of the types of cells so far tested. J. Med. Invest. 46 : 43-47, 1999

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) carries several auxiliary genes not found in the other simple retroviruses (1). Of the six auxiliary genes, four accessory genes designated *nef, vif, vpr,* and *vpu* are dispensable for viral replication at least in certain types of cells (2-5). However, the products of these accessory genes certainly modulate virus replication in tissue culture systems, and more importantly, virus replication and pathogenesis *in vivo*. Of the four accessory proteins, Vpu is always non-essential for virus replication in any kinds of cells tested (6-11), although it has been demonstrated to enhance virion release from cells of various types (8, 12). Even in natural target cells such as peripheral blood mononuclear cells (PBMCs), *vpu* mutant viruses grow quite well (9, 11). It is possible, however, that the results described above may not be general, and may be dependent on the *vpu* mutant clones (10) and cells used in the experiments. It has been reported that *vpu* mutants grow very poorly in macrophage cultures (7, 9).

In this study, a *vpu* mutant, which lacks the expression of the entire *vpu* gene, was newly constructed and monitored for its replication in various types of cells in comparison with another mutant expressing a truncated form of Vpu (13) and a wild-type (wt) clone. We show here that this new *vpu* mutant does replicate in two lymphocytic cell lines and in all PBMC cultures of six individuals, thus demonstrating the dispensability of the HIV-1 Vpu for virus replication in lymphocytic cells.

MATERIALS AND METHODS

Cells, transfection, and infection

A human cervical carcinoma cell line, HeLa (ATCC CCL2), was maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum. CD4-positive human lymphocytic cell lines A3.01 (14) and CEMx174 (15) were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum. Human PBMCs were prepared and cultured as previously described (16). For transfection, uncleaved plasmid DNA was introduced into HeLa cells by the calcium-phosphate coprecipitation method (17). Cells were infected with cell-free virus samples prepared from transfections as previously described (14, 18).

RT assay

Virion-associated reverse transcriptase (RT) activity was measured as described previously (19).

Western blotting

Western immunoblotting was performed as previously described (19). MULTI GEL system (Daiichi Pure Chemicals) was used for polyacrylamide gel electrophoresis. Specific viral Vpu protein was detected by the ECL system (Amersham) using an antibody, a generous gift from Dr. Klaus Strebel, raised against amino acids 33-81 of the HIV-1 Vpu (C-terminus) (20). A serum of an individual infected with HIV-1 was also used for detection of viral structural proteins.

DNA constructs

Molecular clones of HIV-1 designated pNL432 and pNL-Ss have been previously described (8, 13, 17, 21). A *vpu* mutant clone pNL-E65, which lacks the initiation codon (AGG instead of ATG), was constructed by the LA PCR *in vitro* Mutagenesis Kit (Takara). The structure of this mutant was confirmed by dideoxy sequencing.

RESULTS

Western blot analysis of vpu mutants

Our previous genetic studies (8, 11, 13, 21) involved the use of a mutant, NL-Ss, which carries a linker insertion resulting in a translational frameshift after the first 32 amino acids of Vpu like a mutant designated Vpu35 (10, 22) and therefore has the potential to express the entire N-terminal transmembrane (TM) domain (22). We constructed a second HIV-1 *vpu* mutant, NL-E65, which lacks the initiation codon and is therefore unable to express any Vpu-specific sequence. The parental clone of the two *vpu* mutants was T-cell tropic pNL432 (17). We first confirmed the inability of our new *vpu* mutant NL-E65 to express the Vpu protein. HeLa cells were transfected with various clones, and cell lysates were made at 2 days post-transfection. The expression of Vpu was monitored by Western blotting analysis. As shown in Fig. 1A, the two *vpu* mutants were able to produce viral structural proteins normally upon transfection. In contrast, as shown in Fig. 1B, the mutants did not generate Vpu detectable by the anti-Vpu antiserum, as was expected.
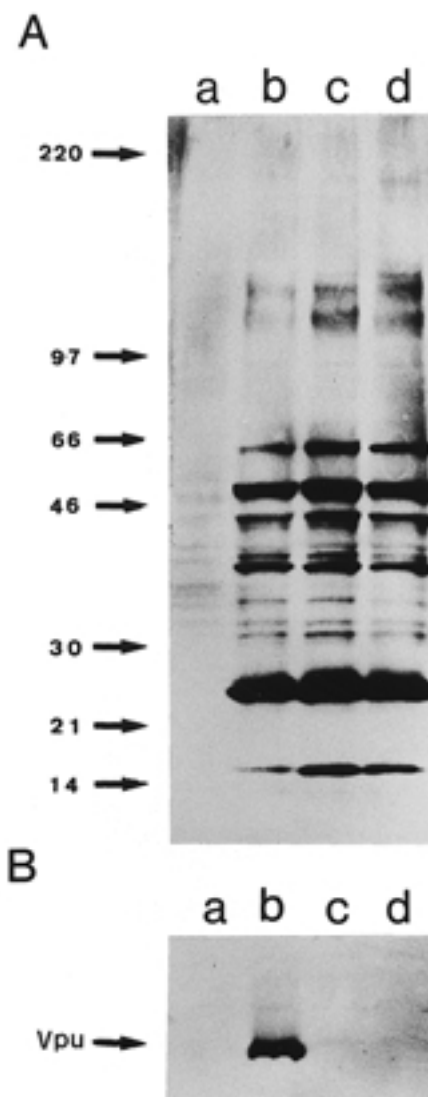

Fig. 1. Western blot analysis of HIV-1 *vpu* mutants. HeLa cells were transfected with a negative control pUC 119 (a), wt pNL432 (b), mutant pNL-Ss (c), or mutant pNL-E65 (d), and cell lysates were made 48 hr later. The expression of structural proteins (A) and of Vpu protein (B) was monitored by Western blot analysis using a serum from an HIV-1 infected individual (A) and an antiserum raised against C-terminal portion of Vpu (B). Note the presence of HIV-1 Env (gp160, gp120), Pol (p66), Gag (p55, p24, p17) in lanes b to d in (A). Molecular weights in kDa are indicated on the left.

Growth property of vpu mutant viruses in established cell lines

When inoculated into CD4-positive cell lines, HIV-1 *vpu* mutant viruses display unique and characteristic growth curves (6, 8, 12, 22). Namely, the growth kinetics of the *vpu* mutant virus are similar to those of wt virus but the expression of virus production in culture medium is much lower than that of wt virus.

We initially examined the growth property of a new HIV-1vpu mutant virus derived from transfection of plasmid pNL-E65 in two CD4-positive cell lines. HeLa cells were transfected with various clones, and cell-free virus samples were prepared at 2 days post-transfection. As shown in Fig.2, in A301 and CEMx174 cells, both vpu mutants, NL-Ss (8, 13) and NL-E65, showed the growth characteristics typical to the vpu mutant.

Growth property vpu mutant viruses in PBMCs

To determine whether Vpu is critical for the replication of HIV-1 in natural target cells, we monitored the growth potential of the NL-E65 vpu mutant virus in stimulated and unstimulated PBMCs. Our previous study using a vpu mutant of macrophage tropic clone has shown that Vpu is quite important for virus replication in macrophages (7). However, only a mild effect of vpu mutation on virus replication was observed in PBMCs, (7, 11).

Cell-free virus samples were prepared as described above, and inoculated into PBMCs prepared from six healthy individuals. We were especially interested in infection of unstimulated PBMCs with NL-E65. It has been recently reported that the accessory protein Nef is very important for virus replication in the unstimulated cells (18). As shown in Fig.3, in stimulated PBMCs, both vpu mutants grew considerably well relative to wt virus in the all six independent preparations as reported previously (11). This was the case in most unstimulated PBMC preparations, as shown in Fig.4. Although the peak level of virus production varied, the two vpu mutants grew relatively well. In one unstimulated PBMC preparation, the two vpu mutants, especially NL-E65, grew very poorly relative to wt virus (Fig.4B).
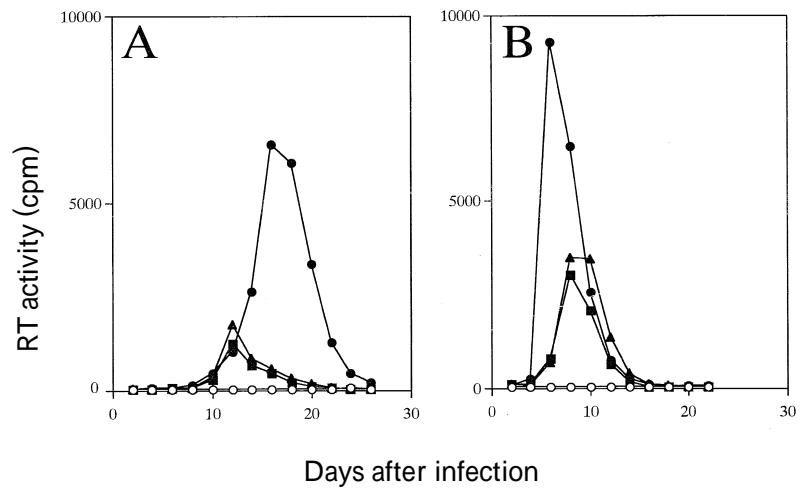
DISCUSSION

The major conclusion of this study is

Fig.2. Growth property of HIV-1 vpu mutants in A301 and CEMx174 cell lines. A301 (A) and CEMx174 (B) cells (10^5) were infected with 4×10^5 RT units of cell-free virus samples prepared from transfected HeLa cells, and RT production in the culture media was determined at the designated intervals. Symbols: , mock-infection; , NL432 (wt) ; , NL-Ss (vpu mutant) ; , NL-E65 (vpu mutant).
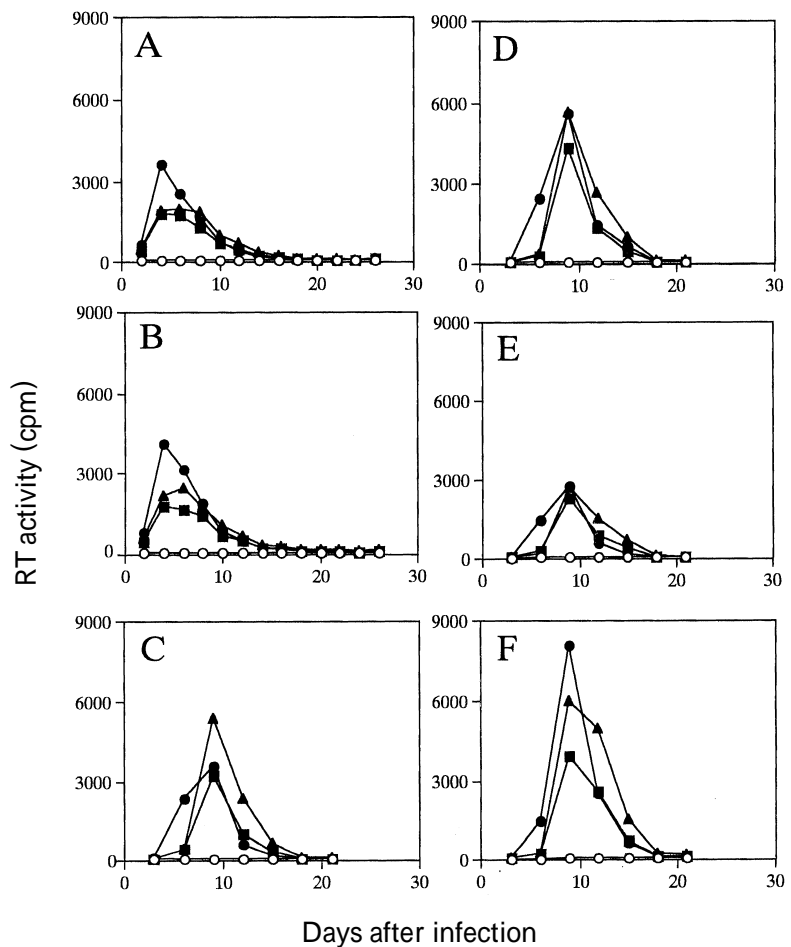

Fig.3. Growth property of HIV-1 vpu mutants in stimulated PBMCs. Stimulated PBMCs were prepared and infected with the virus essentially as described previously (cells were isolated and activated with PHA-P before infection) (18). Cells (10^6) were infected with 10^5 RT units of cell-free virus samples prepared from transfected HeLa cells, and RT production in the culture media was determined at the designated intervals. Symbols: , mock-infection; , NL432 (wt) ; , NL-Ss (vpu mutant) ; , NL-E65 (vpu mutant).

that the HIV-1 Vpu is dispensable for virus replication in any types of CD4-positive cells. Although a critical requirement of Vpu for HIV-1 growth in macrophages has been reported previously (7, 9), HIV-1 lacking the entire *vpu* gene could replicate to some extent in all the cell types tested so far. Whether the Vpu is essential for HIV-1 replication in cells other than those examined to date needs to be determined. It is also possible that Vpu plays an important functional role for *in vivo* replication and pathogenesis like Nef and Vpr (2-5).

It has been reported that the HIV-1 Vpu has two structural domains which are important for two distinct biological activities (10). The TM and cytoplasmic regions represent active domains for the regulation of virus release from cells and the CD4 degradation in cells, respectively. The results of our experiments described here suggest that the TM region of the Vpu does not contribute much to the growth property of the *vpu* mutant. Further genetic study is required to determine the functional domains of HIV-1 Vpu.
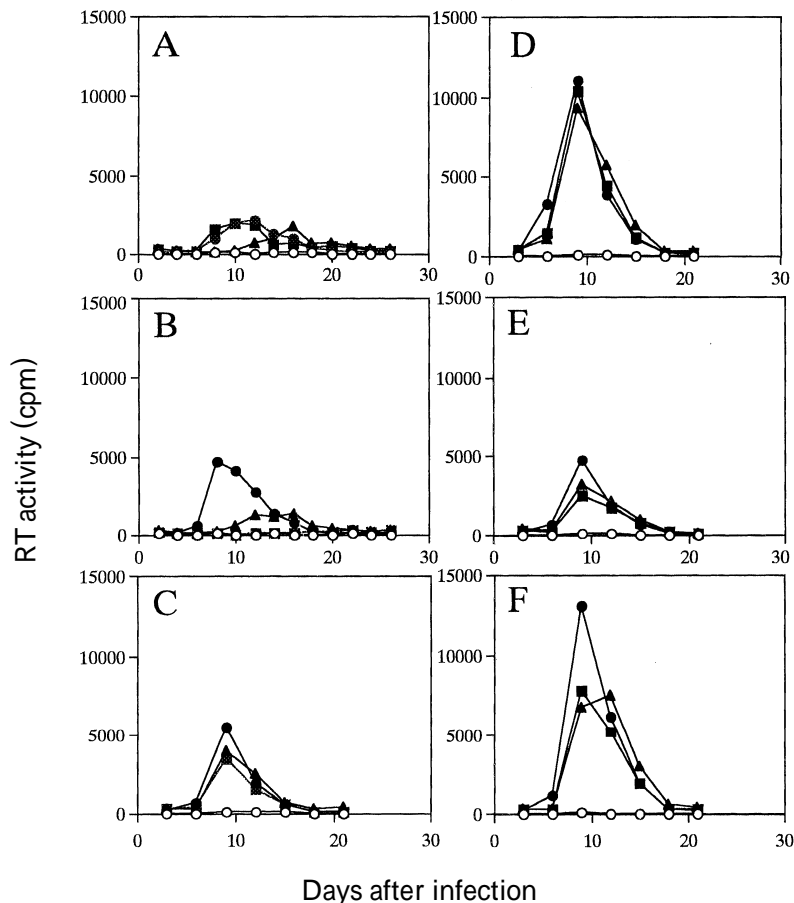
ACKNOWLEDGMENTS

Days after infection

Fig. 4. Growth property of HIV-1 *vpu* mutants in unstimulated PBMCs. Unstimulated PBMCs were prepared and infected with virus essentially as described previously (cells were isolated and virus-infected before activation with PHA-P on the next day) (18). Cells (10^6) were infected with 10^5 RT units of cell-free virus samples prepared from transfected HeLa cells, and RT production in the culture media was determined at the designated intervals. Symbols: , mock-infection; , NL432 (wt); , NL-Ss (*vpu* mutant); , NL-E65 (*vpu* mutant). In panel B, the culture media from cells infected with NL-E65 were clearly RT-positive on days 18 to 26 (3- to 5-fold higher RT counts than those in mock-infected cells).

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