Pseudotyping human immunodeficiency virus type 1 by vesicular stomatitis virus G protein does not reduce the cell-dependent requirement of Vif for optimal infectivity: functional difference between Vif and Nef

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The functions of Vif and Nef in human immunodeficiency virus type 1 (HIV-1) infection have some similarities: Vif- and Nef-dependent enhancement of HIV-1 replication is cell type-specific, and defective mutations in these genes result in restricted proviral DNA synthesis in infected cells. It has recently been shown that pseudotyping HIV-1 by the envelope glycoprotein of vesicular stomatitis virus (VSV-G) targets HIV-1 entry to an endocytic pathway and suppresses the requirement of Nef for virus infectivity. In this study, we examined whether VSV-G pseudotyping suppresses the requirement of Vif for HIV-1 infectivity. It was found that pseudotyping HIV-1 by VSV-G did not compensate for the Vif function. Together with the findings that Vif does not influence virus binding/entry and virion incorporation of Env, it is concluded that Vif enhances HIV-1 infectivity at the post-entry step(s) independently of the Env function by a different mechanism to that of Nef.

Human immunodeficiency virus type 1 (HIV-1) encodes four accessory genes, vif, vpr, vpu and nef, in addition to structural gag, pol, and env genes and regulatory tat and rev genes. Among the accessory genes, vif is essential for HIV-1 replication in non-permissive cells such as primary lymphocytes/macrophages and some cell lines (Akari et al., 1992; Borman et al., 1995; Courcoul et al., 1995; Fan & Peden, 1992; Fisher et al., 1987; Gabuzda et al., 1992; Sakai et al., 1993; Simon et al., 1995; Simon & Malim, 1996; Sodroski et al., 1986; Sova & Volsky, 1993; Strebel et al., 1987; von Schwedler et al., 1993). The function of Vif is dependent on the producer cell type; recent reports have demonstrated that an unknown endogenous inhibitor for HIV-1 infection is present in non-permissive cells and Vif protein inhibits the function of this factor (Madani & Kabat, 1998; Simon et al., 1998). The vif gene-defective virus produced from non-permissive cells (∆Vif) shows negligible infectivity in the early phase of HIV-1 infection (Borman et al., 1995; Courcoul et al., 1995; Simon & Malim, 1996). The defective infectivity of ∆Vif is, at least, due to inefficiency of synthesizing proviral DNA in infected cells (Chowdhury et al., 1996; Courcoul et al., 1995; Reddy et al., 1995; Schwedler et al., 1993; Simon & Malim, 1996; Sova & Volsky, 1993). It remains unclear which step(s) from virion binding to cells to reverse transcription in the early stage of ∆Vif infection is abrogated.

The function of Nef protein in the early stage of the HIV-1 replication cycle has some similarities with that of Vif; Nef-dependent enhancement of HIV-1 replication is cell type-dependent (Miller et al., 1994; Spina et al., 1994; Tokunaga et al., 1998a, b) and defective mutations in the gene result in restricted proviral DNA synthesis in infected cells (Aiken & Trono, 1995; Schwartz et al., 1995). Recent reports (Aiken, 1997; Luo et al., 1998) have shown that pseudotyping HIV-1 by the envelope glycoprotein of vesicular stomatitis virus (VSV-G) targets HIV-1 entry to an endocytic pathway and suppresses the requirement of Nef for infectivity. These results suggest that Nef acts not only on post-entry step(s) such as uncoating and reverse-transcription but also on binding-entry step(s), which can be bypassed through an endocytic pathway. Actually, we have recently reported the cell-dependent requirement of Nef for efficient entry of HIV-1 (Tokunaga et al., 1998b).

In this study, we examined whether the endocytic pathway induced by VSV-G pseudotyping suppresses the requirement of Vif for virus infectivity as well as Nef. We also evaluated the effects of Vif on the efficiency of virus binding and entry, virion incorporation of Env, and processing of Gag proteins.

First, to determine the effect of VSV-G pseudotyping on the ability of Vif to enhance infectivity of HIV-1 produced in
non-permissive cells, HIV-1 particles defective in either Env (ΔEnv) or both Vif and Env (ΔVifΔEnv) pseudotyped by VSV-G were made. As a control, the envelope protein of amphotropic murine leukaemia virus (A-MLV-Env), which uses the membrane fusion pathway for virus entry like HIV-1, was used for pseudotyping. Infectious molecular clones of HIV-1 pNL-432 and its Vif- Env- and Nef-defective mutants, pNL-Nd, pNL-Kp and pNL-M1T, respectively, have been described previously (Adachi et al., 1986, 1991) or elsewhere (H. Akari, S. Arold, T. Fukumori, T. Okazaki & A. Adachi, unpublished results). To make a Vif/Env or a Env/Nef double-defective mutant pNL-NdKp or pNL-KpM1T, respectively, a frameshift mutation was introduced into the KpnI site of the env gene in pNL-Nd and pNL-M1T as previously described (Adachi et al., 1991). A VSV-G expression construct pCMV-G (Yee et al., 1994) and an A-MLV-Env expression plasmid SA-A-MLV-env (Page et al., 1990) were kindly provided by A. Miyanohara (University of California, San Diego) and D. Littman (New York University Medical Center, Skirball Institute, New York), respectively. To obtain wild-type (WT), ΔVifΔEnv or ΔNef virus from non-permissive cells, 10 µg of plasmid pNL-432, pNL-Nd, pNL-Kp or pNL-M1T was transfected to 4× 10⁸ H9 cells (Popovic et al., 1984) by electroporation at 0.2 kV on a Gene Pulser (Bio-Rad). For preparation of pseudotyped viruses, 10 µg of pNL-Kp, pNL-NdKp or pNL-KpM1T and pCMV-G or SA-A-MLV-env were co-transfected into H9 or HeLa cells. The culture supernatants were harvested 24 h after electroporation, filtered through a 0.45 µm filter, and aliquoted at −80 °C. Quantification of the amounts of virus was conducted by a p24 antigen ELISA kit (Cellular Products). At 24 h post-electroporation, no clear differences in the amount of p24 antigen in the culture supernatants were observed among any combination of plasmids (data not shown). The infectivity of the viruses was measured by MAGI assay as previously described (Kimpton & Emerman, 1992). As shown in Fig. 1(A), ΔVif showed 20-fold lower infectivity than WT virus as previously described (Fouchier et al., 1996). Interestingly, pseudotyping HIV-1 by not only A-MLV-Env but also VSV-G did not restore the lower infectivity of the Vif-defective mutant (Fig. 1 B). On the other hand, ΔNef exhibited 10-fold lower infectivity than WT virus, and pseudotyping HIV-1 by VSV-G suppressed the requirement of Nef as previously reported (Aiken, 1997; Luo et al., 1998) (Fig. 1 C). These results indicated that the infectivity enhancement by Vif is independent of the entry pathway and suggested that Vif function is unrelated to envelope glycoprotein coating on the virion.

Next, we determined the effect of Vif on the ability of viruses to bind to and enter into the target cells. Virus binding and entry assay was performed as previously described (Adachi et al., 1996; Akari et al., 1993) with minor modifications. An equal amount of viruses (corresponding to 5 ng of p24) was pelleted by ultracentrifugation at 20,000 g for 2 h at 4 °C, and resuspended in RPMI-1640 medium with 10% foetal bovine serum. The concentrated viruses were mixed with 5 × 10⁶ H9 cells, followed by incubation at 4 °C for 1 h with occasional agitation. Cells were then washed three times and were lysed to measure the amount of viruses bound to the cells by p24 ELISA assay. To monitor entry of the viruses, the cell-binding cells were washed three times and were incubated at 37 °C for 1 h. Cells were then treated with PBS with 0.05% trypsin and 50 mM EDTA at 37 °C for 5 min. They were washed once and then were lysed to measure the amount of virus in the cells as mentioned above. As shown in Fig. 2, no major differences in the efficiency of virus binding and entry between WT and ΔVif were observed.

The results described above strongly suggested that Vif protein scarcely affects the incorporation of Env into the virion. To ascertain this, WT and ΔVif viruses obtained from non-permissive and permissive cells by electroporation were analysed by Western blotting. Virions were pelleted by ultracentrifugation through 20% sucrose cushions at 20,000 g for 2 h at 4 °C. Lysates of virions and cell pellets were
subjected to electrophoresis through an SDS-gradient polyacrylamide gel, and the separated proteins were blotted onto nitrocellulose membranes. The membranes were treated with antibodies specific to HIV-1 proteins, and visualized by an ECL system (Amersham). The anti-gp160 and anti-p17 Gag polyclonal antibodies were provided by M. Page and G. Reid, respectively, through MRC AIDS Directed Programme Reagent Project. The anti-Vif C terminus peptide antibody was prepared in our laboratory. To adjust the amount of virus antigens, virus and cell lysates were quantified for the amount of p24 core antigen and an appropriate volume of each lysate was used. It was confirmed that non-permissive H9 and permissive M8166 cells (Shibata et al., 1991) transfected with pNL-432 but not with pNL-Nd expressed Vif protein (Fig. 3 A). As expected, the level of Env in ΔVif was comparable to that in WT virus, irrespective of the permissivity of the producer cells (Fig. 3 B, upper panels). The expression level of Env was indistinguishable between cells transfected with pNL-432 and pNL-Nd (Fig. 3 B, lower panels). In addition, Gag proteins in the virions and cells were analysed. As shown in Fig. 3 (C), no clear differences with respect to the processing of Gag proteins in virions and cells were observed for WT and ΔVif.

In this study, we evaluated the functional role of Vif protein in the early phase of HIV-1 infection. Like Vif, Nef enhances virion infectivity in a cell-dependent manner (Miller et al., 1994; Spina et al., 1994; Tokunaga et al., 1998 a, b) and deletion in the nef gene results in the restricted proviral DNA synthesis (Aiken & Trono, 1995; Schwartz et al., 1995). On the other hand, our present findings were in contrast to the recent reports that pseudotyping HIV-1 by VSV-G targets HIV-1 entry to an endocytic pathway and suppresses the requirement of Nef for infectivity (Aiken, 1997; Luo et al., 1998). Neither AMLV-Env nor VSV-G pseudotyping rescued the defective infectivity of ΔVif virus (Fig. 1). This finding indicates that the requirement of Vif is not bypassed through an endocytic

Fig. 2. Effect of Vif on the efficiency of HIV-1 binding and entry to H9 cells. H9 cells (5 x 10⁵) were treated with an equal amount (5 ng p24) of HIV-1 WT, ΔVif or ΔEnv produced in H9 cells electroporated with 10 µg of various plasmid clones indicated. The amounts of viruses binding to cells (A) and those entered into cells (B) were quantified by p24 antigen ELISA assay as described in the text. Representative results of three independent experiments are shown.

Fig. 3. Western blotting analysis of HIV-1 proteins. H9 and M8166 cells were electroporated with 10 µg of pUC19 (mock), pNL-432 or pNL-Nd. At 24 h post-electroporation, the culture supernatants and cells were harvested for the preparation of lysates for Western blotting analysis. Each sample contained 5 ng p24 antigen. Vif protein in the cells (A), and Env (B) and Gag (C) proteins in the virions and cells are shown.
pathway and that Vif and Nef proteins use different mechanisms for enhancing virus infectivity.

The present results in this study indicated that Vif does not influence the entry step(s) of HIV-1 in the replication cycle (Figs 1 and 2). Together with the consensus finding that ΔVif is defective for proviral DNA synthesis in the infected cells (Chowdhury et al., 1996; Courcoul et al., 1995; Goncalves et al., 1996; Reddy et al., 1995; Schwedler et al., 1993; Simon & Malim, 1996; Sova & Volsky, 1993), it can be concluded that the ΔVif mutation reduces the infectivity due to the defect(s) in a step(s) from uncoating to reverse transcription.

Our data revealed that no envelope glycoprotein among HIV-1, VSV and A-MLV is associated with the function of Vif in non-permissive cells. It has been shown that HIV-1 matrix (MA) protein directly associates with the cytoplasmic tail of Env gp41 and that a mutation in MA results in the loss of Env incorporation into the virion (Cosson, 1996; Freed & Martin, 1995). Furthermore, truncation of the C-terminal 144 amino acids of gp41 results in sufficient infectivity without association of gp41 with MA (Reil et al., 1998). It is possible that the Vif defect affects the function of MA, resulting in inefficient incorporation of Env into virions. To test this possibility, we examined whether a truncation of the cytoplasmic tail of Env gp41 rescues the Vif defect. The truncation did not compensate for the defective phenotype of ΔVif (H. Akari & A. Adachi, unpublished results). This result also supports our conclusion that the function of Vif is unrelated to Env.

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References


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