ORIGINAL

Different interaction between HIV-1 Vif and its cellular target proteins APOBEC3G/APOBEC3F

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Abstract : We examined a series of site-directed point mutants of human immunodeficiency virus type 1 (HIV-1) Vif for their interaction with cellular anti-viral factors APOBEC3G/APOBEC3F. Mutant viruses that display growth-defect in H9 cells did not counteract effectively APOBEC3G and/or APOBEC3F without exception, as monitored by single-cycle infectivity assays. While growth-defective mutants of Vif C-terminal region were unable to suppress APOBEC3G/APOBEC3F, some N-terminal region mutants did neutralize one of APOBEC3G/APOBEC3F. These data have suggested that members of APOBEC3 family other than APOBEC3G/APOBEC3F are not important for anti-HIV-1 activity. Furthermore, APOPEC3G/APOBEC3F were found to differently associate with Vif in virions as analyzed by equilibrium density centrifugation. Taken together, these results indicated that interaction of HIV-1 Vif and APOBEC3G is distinct from that between Vif and APOBEC3F. J. Med. Invest. 57 : 89-94, February, 2010

Keywords **:** *HIV-1, Vif, APOBEC3G, APOBEC3F*

INTRODUCTION

The genome of HIV-1 encodes Vif as one of accessory viral proteins that are unique to primate immunodeficiency viruses. HIV-1 Vif efficiently neutralizes the strong anti-retroviral effects of APOBEC3G/APOBEC3F in natural target cells by targeting them to proteasomal degradation (1). Through this activity of Vif, HIV-1 can grow in T lymphocytes and macrophages, the two major targets cells of the virus. Extensive studies by us and others have revealed that HIV-1 Vif consists of two major functional domains with various motifs (2-4). HIV-1 Vif binds to APOBEC3G/APOBEC3F by its N-terminal region (2-10), and degrades them through interaction with its C-terminal region (2, 11-14). While Vif mediates proteasomal degradation of both APOBEC3 proteins, it binds to them differently via several distinct motifs (2-10). In some $CD4^+$ cells such as H9, primary T lymphocytes, and macrophages that are non-permissive for HIV-1 without Vif, APOBEC3G/APOBEC3F are found to act against HIV-1. However, biological and biochemical basis for interaction of Vif and APOBEC3G/APOBEC3F is not well understood yet.

APOBEC3G/APOBEC3F belong to a family of polynucleotide cytidine deaminases with diverse functions (1). Although it has been well established that APOBEC3G/APOBEC3F are potent antiretroviral cellular factors, several other APOBEC family members have been reported to be able to inhibit HIV-1 (1). However, the extent of contribution by these APOBEC proteins to block or impede HIV-1 replication remains to be determined.

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In this study, we have determined a single-cycle viral infectivity of numerous HIV-1 *vif* mutants in the presence of APOBEC3G or APOBEC3F, and compared the results with growth characteristics of the mutants in non-permissive H9 cells. In addition, we have examined status of Vif/APOBEC3 complexes in cells and virions by equilibrium density centrifugation. We demonstrate here the distinct interaction of Vif and the two APOBEC3 proteins.

MATERIALS AND METHODS

Cells

Human monolayer cell lines 293T (15) and MAGI (16) were maintained in Eagles's minimal essential medium containing 10% heat-inactivated fetal bovine serum.

Transfection

Sub-confluent 293T cells in 90 mm dishes were co-transfected with a proviral clone and a flag-tagged expression vector of APOBEC3G or APOBEC3F (3, 4) by the calcium-phosphate co-precipitation method (17). On day 2 post-transfection, cells and cell-free virus samples were prepared as previously described (3, 4, 17). Viral amount was determined by the HIV-1 p24 antigen ELISA kit (ZeptoMetrix Corp.).

MAGI assay

Viral single-cycle infectivity per 0.1 ng of Gag-p24 was determined by the MAGI assay as previously described (16).

Analysis of Vif and APOBEC3G/APOBEC3F complexes in cells and virions

Transfected 293T cells and virions produced in the cells were lysed in a lysis buffer (1% Nonidet P-40, 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA and 1% protease inhibitor cocktail (Sigma-Aldrich)). For preparation of virions, culture supernatants from transfected 293T cells were filtered through 0.45-μm filters, and partially purified by ultracentrifugation through 25% sucrose for 2 h at 80,000 $\times g$ using SW41 rotor. Cell and virion lysates were resolved by SDS-PAGE, and electrophoretically transferred to polyvinilidene fluolide membranes. Membranes were treated with anti-FLAG (Sigma-Aldrich), anti-Vif (NIH AIDS Research and References Reagent Program) or anti-p24 (18) antibody, and visualized by ECL plus Western blotting detection system (Amersham Pharmasia Biotech Inc.). For analysis by equilibrium density centrifugation, cell and virion lysates were loaded on the top of 5-50% sucrose gradient, and spun for 16 h at 100,000 $\times g$ using SW41 rotor. Eight fractions were collected from each tube, and precipitated by trichloroacetic acid. Samples were then analyzed by immunoblotting as described above.

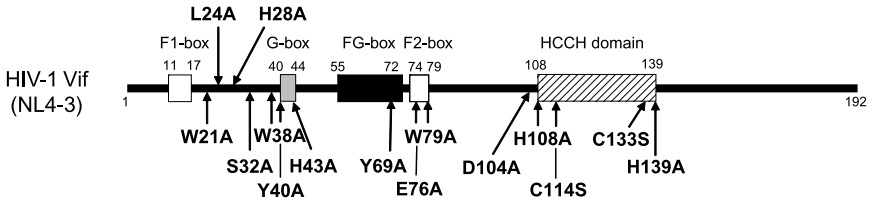
DNA constructs

Proviral *vif* mutant clones of HIV-1 NL4-3 have been previously described (3, 4, 19).

RESULTS AND DISCUSSION

We have previously generated a series of HIV-1 *vif* mutants from wild-type (WT) NL4-3 clone, and analyzed them biologically and biochemically (Fig. 1) (3, 4, 19). Approximately half of the site-directed point mutants were defective for virus growth in non-permissive H9 cells, and were predicted to be susceptible to anti-viral activity of APOBEC3 proteins. In fact, mutants designated W21A, S32A, W38A, Y69A and H108A did not overcome the anti-HIV-1 action of APOBEC3G/APOBEC3F (3, 4). Moreover, while mutants Y40A and H43A did not neutralize the activity of APOBEC3G, E76A and W79A did not suppress APOBEC3F (3, 4).

In order to do a more systemic genetic analysis of anti-APOBEC activity of HIV-1 Vif, we selected fifteen clones from our proviral mutants (3, 4, 19) as shown in Fig. 1. These included mutants carrying mutations in known functional motifs or outside the regions. A mutant without Vif (ΔVif) did not grow at all in H9 cells (Fig. 1) (3, 19). The other mutants except for H28A were growth-defective in H9 cells to some extent (Fig. 1) (3, 19). The mutants and expression vectors of APOBEC3G or APOBEC3F were co-transfected into 293T cells, and equal amounts of resultant viruses as determined by Gag-p24 ELISA were assayed for the single-cycle infectivity in MAGI cells. All the mutants were simultaneously examined for infectivity in each experiment to know relative anti-APOBEC activity. Fig. 2 shows the MAGI infectivity of various viruses derived from proviral clones (Fig. 1). In the presence of APOBEC3G and APOBEC3F, ΔVif mutant displayed less than 20% and 30% infectivity in a single-replication cycle, respectively, relative to that of WT virus. As expected, L24A and H28A, mutants with almost normal growth phenotype in



Virus clone	Growth in H9 cells	Virus clone	Growth in H9 cells
WT	Wild type	Y69A	No
Δ Vif	No	E76A	Poor
W21A	No	W79A	No
L24A	Slightly poor	D104A	Poor
H28A	Wild type	H108A	No
S32A	No	C114S	No
W38A	No	C133S	No
Y40A	No	H139A	No
H43A	Poor		

Figure 1 : Major functional domains of HIV-1 Vif protein and its mutants used in this study. Functionally important domains or motifs in Vif (2-4), and positions and designations of the mutants are indicated at the upper part. All mutants are derived from HIV-1 NL4-3 clone (17) (GenBank accession no. AF324493). At the lower part, growth property of mutant viruses in non-permissive H9 cells (3, 19) are summarized as follows. Wild type, virus growth similar to that of wild type virus ; No, no detectable virus growth ; Slightly poor, virus growth kinetics are similar to those of wild type virus but virion production at the peak is around 50% of that by wild type virus ; Poor, virus growth kinetics are significantly delayed relative to those of wild type virus (at least 3 days). Abbreviations : WT, wild-type virus NL4-3 ; ΔVif, Vif-minus virus NL-Nd (22).

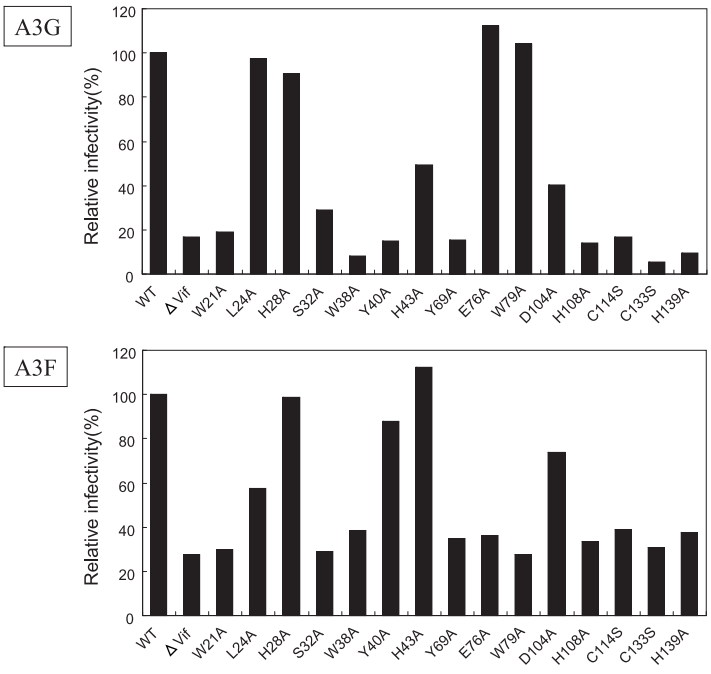


Figure 2 : Single-cycle infectivity of various *vif* mutants in the presence of APOBEC3G/APOBEC3F. Virus samples were prepared from 293T cells co-transfected with a proviral clone (15 μg) and an expression vector of flag tagged APOBEC3G/APOBEC3F (1 μg). Single-cycle infectivity was determined by MAGI assay (16), and viral infectivity relative to that of WT NL4-3 clone is shown. A3G, APOBEC3G ; A3F, APOBEC3F ; WT, wild-type virus NL4-3 ; ΔVif, Vif-minus virus NL-Nd (22).

H9 cells (Fig. 1) (3), behaved similarly with WT virus in the presence of APOBEC3G/APOBEC3F. However, L24A was somewhat sensitive to A3F activity in agreement with its slightly poor growth in H9 cells (Fig. 1) (3). As can be easily seen in Fig. 2, mutants other than L24A and H28A showed considerable defects against APOBEC3G, APOBEC3F, or both. Following mutants are typical examples :

Y40A/APOBEC3G, W79A/APOBEC3F, H108A/APOBEC3G and APOBEC3F. The experiment was repeated with similar results. In summary, Vif mutants with a growth-defect in H9 cells were always susceptible to anti-viral activity of APOBEC3G and/or APOBEC3F to a various extent as shown in Table 1. Of note, viral ability to neutralize APOBEC3G/APOBEC3F paralleled perfectly with viral growth property in H9 cells (Fig. 1).

Table 1. Ability of various *vif* mutants to neutralize APOBEC3G/APOBEC3F

Mutant	A3G	A3F
W21A	-	-
L24A	++	+
H28A	++	++
S32A	-	-
W38A	-	-
Y40A	-	++
H43A	+	++
Y69A	-	-
E76A	++	-
W79A	++	-
D104A	+	+
H108A	-	-
C114S	-	-
C133S	-	-
H139A	-	-

A3G, APOBEC3G ; A3F, APOBEC3F.
++, Wild type ability ; +, Partially defective ; -, No ability.

It has been reported that APOBEC3G/APOBEC3F are present in high molecular mass (HMM) complexes in some cells such as immortalized or activated primary T cells and that they reside in low molecular mass (LMM) complexes in the resting primary T cells (20, 21). It has also been shown that only LMM complexes exhibit the cytidine deaminase activity *in vitro* (20, 21). These findings prompted us to examine whether HIV-1 Vif is associated with the protein complexes of APOBEC3G/APOBEC3F. We employed equilibrium density centrifugation to segregate HIV-1 Vif and APOBEC3G/APOBEC3F complexes. As a control Vif mutant, H108A, which can bind to both of APOBEC3G/APOBEC3F (3, 4) and are unable to neutralize APOBEC3G/APOBEC3F (Table 1), was chosen and used in this experiment. Cell and virion lysates were prepared from 293T cells co-transfected with an expression vector for APOBEC3G/APOBEC3F and WT or H108A mutant proviral clone. Lysates were then centrifuged onto 5-50% sucrose gradient, and samples collected from centrifugal tubes were subjected to immunoblot analysis. As shown in Fig. 3, in the absence of Vif, while Gag-p24 was mostly observed in low density fractions in virus-producer cells and virions, APOBEC3G/APOBEC3F were mainly present in high density fractions in

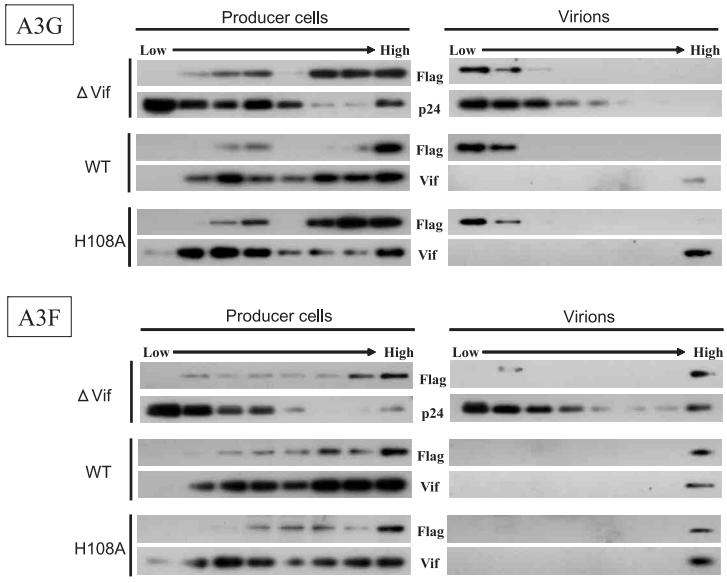


Figure 3 : Profile of Vif and APOBEC3G/APOBEC3F complexes in producer cells and progeny virions as revealed by equilibrium density centrifugation. Cell and virion lysates were prepared from 293T cells co-transfected with a proviral cloned and an expression vector of APOBEC3G/APOBEC3F as described in the legend to Fig. 2, and were ultracentrifuged onto 5-50% sucrose gradient. Eight fractions collected from each tube were then analyzed by immunoblotting. WT, wild-type virus NL4-3 ; ΔVif, Vif-minus virus NL-Nd (22) ; Low, low density ; High, high density.

the cells. In contrast, in virions, APOBEC3G and APOBEC3F were found to predominantly exist in LMM and HMM fractions, respectively. We also examined APOBEC3G/APOBEC3F protein complexes in the presence of WT and H108A Vif. WT and H108A Vif did not affect the APOBEC3G/APOBEC3F complexes in cells and virions significantly (panels WT-Flag and H108A-Flag in Fig. 3). However, in producer cells, the mutation of His108 partially shifted the Vif complexes from high to low density (panels WT-Vif and H108A-Vif in Fig. 3). Whether this small mutational effect is directly related to the inability of H108A-Vif to neutralize APOBEC3G/APOBEC3F remains to be determined. If related, the molecular basis for the phenotype should be studied in detail. A systematic analysis of the mutants other than H108A would give a more definitive answer. In virions, WT and H108A Vif similarly formed HMM protein complexes. In summary, the results described above suggested that HIV-1 Vif interacts APOBEC3G/APOBEC3F in cells, and that Vif may be contained in the APOBEC3F HMM complex in virions.

In this study, we have demonstrated by using a variety of HIV-1 *vif* mutant viruses that APOBEC3G/APOBEC3F are essential to restrict viral replication in target cells. All growth-defective mutants are always sensitive to the strong anti-viral effect mediated by APOBEC3G and/or APOBEC3F (Fig. 1, Fig. 2 and Table 1). This study has clearly indicated that HIV-1 Vif interacts with APOBEC3G/APOBEC3F differently. As illustrated in Fig. 1, there are several motifs in HIV-1 Vif for binding to APOBEC3G, APOBEC3F, or both. We confirmed previous results and extended the observations reported. Importantly, there may be another domain around the site of W21-L24-S32-W38 for binding to both APOBEC proteins (FG-box), not reported yet.

Our analysis of Vif-APOBEC protein complexes here has also suggested that there may be molecular difference between interactions of Vif and the two APOBEC proteins in virions. In virions, while Vif and APOBEC3F was present in HMM fractions, (particularly in the case of H108A Vif), APOBEC3G resided in LMM fractions. In this regard, our finding that H108A Vif does not bind to APOBEC3G in virions but does in cells (4) is interesting. In contrast, WT Vif was shown to bind to APOBEC3G in virions (4). More detailed biochemical studies would reveal molecular structural basis for different interaction of Vif and APOBEC3G/APOBEC3F.

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