Distinct Domains within APOBEC3G and APOBEC3F Interact with Separate Regions of Human Immunodeficiency Virus Type 1 Vif[†]

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Human APOBEC3G (A3G) and APOBEC3F (A3F) inhibit the replication of Vif-deficient human immunodeficiency virus type 1 (HIV-1). HIV-1 Vif overcomes these host restriction factors by binding to them and inducing their degradation. Thus, the Vif-A3G and Vif-A3F interactions are attractive targets for antiviral drug development, as inhibiting these interactions could allow the host defense mechanism to control HIV-1 replication. Recently, it has been reported that amino acids 105 to 156 of A3G are involved in the interaction with Vif; however, to date, the region of A3F involved in Vif binding has not been identified. Using our previously reported Vif mutants that are capable of binding to only A3G (3G binder) or only A3F (3F binder), in conjunction with a series of A3G-A3F chimeras, we have now mapped the APOBEC3-Vif interaction domains. We found that the A3G domain that interacts with the Vif YRHHY region is located between amino acids 126 and 132 of A3G, which is consistent with the conclusions reported in previous studies. The A3F domain that interacts with the Vif DRMR region did not occur in the homologous domain but instead was located between amino acids 283 and 300 of A3F. These studies are the first to identify the A3F domain that interacts with the Vif DRMR region and show that distinct domains of A3G and A3F interact with different Vif regions. Pharmacological inhibition of either or both of these Vif-A3 interactions should prevent the degradation of the APOBEC3 proteins and could be used as a therapy against HIV-1.

The human cytidine deaminases APOBEC3G (A3G) and APOBEC3F (A3F) are members of an innate immune system that inhibit the replication of a wide variety of viruses including murine leukemia virus (2, 10, 14, 22), human T-lymphotropic virus (29, 43), hepatitis B virus (40, 50, 53), and prototype foamy virus (8, 26, 42). Of particular importance is their potent inhibition of Vif-deficient human immunodeficiency virus type 1 (HIV-1) (24, 45, 57, 64), which they achieve through a number of mechanisms, including G-to-A hypermutation (14, 15, 23, 30), the inhibition of cDNA synthesis (1, 18, 27, 33, 47, 55), and the inhibition of integration (27, 33). To counteract the effects of A3G and A3F, the HIV-1 Vif protein induces their degradation (6, 25, 32, 34, 46, 49, 63), thereby preventing them from being packaged into budding virions. Vif acts by forming a bridge between the APOBEC3 proteins and the cellular ubiquitin-dependent proteasomal degradation pathway, resulting in the polyubiquitination and degradation of the APOBEC3 proteins. The ¹⁴⁴SLQYLA¹⁴⁹ domain and the zinc-binding motif, H¹⁰⁸-X₅-C-X₁₇₋₁₈-C-X₃₋₅-H¹³⁹ (HCCH), of Vif have been shown to drive this process by interacting with the cellular proteasomal degradation proteins Elongin C and Cullin 5, respectively (28, 32, 34, 35, 58, 59, 63). In addition, we recently identified the domains of Vif required for interactions with A3G and A3F and unexpectedly found that two distinct domains were involved: the ⁴⁰YRHHY⁴⁴ domain was necessary for interactions with A3G, and the ¹⁴DRMR¹⁷ domain was necessary for interactions with A3F (41). The substitution

of these domains with alanine residues completely abolished Vif function against either A3G or A3F but not both. These results were further supported by evidence that specific Vif mutants (experimental and naturally occurring ones) maintained activity against either A3G or A3F but not both (36, 37, 48, 52). In addition, recent studies by He et al. identified additional domains of Vif, one of which is involved in interactions with both A3G and A3F (V⁵⁵-L⁷²) and another of which is important for binding to A3F (T⁷⁴-W⁷⁹) (16).

Thus, we and others have identified a number of potential targets for the development of antiviral agents. Recent studies support such a strategy by demonstrating that Vif-derived peptides overlapping the A3G-binding region were able to inhibit the Vif-A3G interaction (36). An alternative approach is to target the Vif-binding domains on A3G and A3F. Although a few studies have sought to identify the region of A3G that interacts with Vif (6, 19, 65), to date, the Vif-binding domain of A3F has not been studied. Truncation mutants of A3G identified amino acids 54 to 124 and 105 to 156 as being involved (6, 65); however, the potential loss of the binding determinant through a misfolding of the truncation mutant, as well as the potential for nonspecific binding to misfolded proteins, was not fully addressed. Huthoff and Malim previously employed alanine substitution mutants of full-length A3G clones and identified amino acids 128 to 130 as being involved in Vif binding (19). However, our previous observations that the HIV-1 Vif-resistant D128K mutant of A3G interacted with Vif in coimmunoprecipitation (co-IP) assays suggested that other A3G determinants could also be involved in Vif binding (41, 60).

There is only 49% amino acid identity between human A3G and A3F. Based on this low level of homology and our obser-

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vation that different determinants of Vif are involved in interactions with these two proteins, we hypothesized that structurally distinct domains of A3G and A3F are involved in the interaction with Vif. To test this hypothesis, and thereby identify the A3G and A3F determinants necessary for Vif binding, we assessed the ability of a panel of APOBEC3 chimeras to bind to wild-type (WT) Vif as well as YRHHY>A5 mutant Vif, which binds only to A3F (the 3F binder), and DRMR>A4 mutant Vif, which binds only to A3G (the 3G binder). To avoid the possibility of false-negative or false-positive results due to the misfolding and/or mislocalization of the proteins, only chimeras capable of inhibiting Vif-deficient HIV-1 were used. The results of these studies, in conjunction with additional mutational analyses, showed that amino acids 126 to 132 of A3G are involved in binding to Vif. In support of our hypothesis that distinct determinants of A3G and A3F are involved in Vif binding, we found that amino acids 283 to 300, near the C terminus of A3F, were essential for the interaction with Vif. As previously reported (60, 65), the binding of A3G to Vif was found to be insufficient for the induction of A3G degradation. A larger region of A3G that included amino acids 121 to 163 was needed for degradation upon Vif binding. In contrast, amino acids 283 to 300 of A3F were sufficient for both binding and degradation. Identification of the distinct domains of A3G and A3F that interact with the previously identified determinants of Vif will enable the design of strategies to interfere with these two interactions and thereby prevent HIV-1 replication in cells that express A3G and/or A3F.

MATERIALS AND METHODS

Plasmid construction and cell culture. Chimeric A3G-A3F plasmids with N-terminal FLAG epitope tags were constructed by overlapping PCR using pFLAG-A3G (41) and pFLAG-A3F (41) as starting templates. As an example, the N-terminal portion of Flag-G¹⁻⁹⁹F⁹⁹⁻³⁷³ was amplified from pFLAG-A3G using primers FLAG-APO-f (5'-GATCGCGGCCGCTATGAAGCCTCACTTC AGAAC-3') and G1-99-F99-r (5'-GGCCAGCTTCGCCACACACTTTGTGC AGGG-3'). The C-terminal portion of Flag-G¹⁻⁹⁹F⁹⁹⁻³⁷³ was amplified from pFLAG-A3F using primers G99-F99-f (5'-CCCTGCACAAAGTGTGTGGCG AAGCTGGCC-3') and FLAG-A3F-r (5'-GATCTCTAGATGAGCAGGAG GCTAGAGGAG-3'). The resulting amplicons were then combined in a second-round PCR using outer primers FLAG-APO-f and FLAG-A3F-r. The final product was digested with NotI and XbaI and cloned into NotI- and XbaI-digested pFLAG-A3G, displacing the A3G fragment and replacing it with the newly constructed G¹⁻⁹⁹F⁹⁹⁻³⁷³ fragment. With the exception of the pFLAG-G¹⁻¹⁶³F¹⁵⁹⁻³⁷³, A3G/F126-129, A3G/F126-132, A3G/F126-132*, and A3F/G291/F chimeras, all chimeras were produced in the same manner; the sequences of the primer pairs used for the construction of all the plasmids are available upon request. Plasmid pFLAG-G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ was made by HindIII and XbaI digestion of A3G+F, which was kindly provided by Reuben Harris (13), and by ligation of the GF chimera-containing fragment into HindIII- and XbaI-digested pFLAG-A3G, displacing the A3G fragment and replacing it with the A3G+F fragment. pA3F/G291/F was also produced by overlapping PCR; however, unlike the other chimeras, the starting template was pF¹⁻²⁸²G²⁹¹⁻³⁸⁴.

Plasmids pA3G/F126-129, pA3G/F126-132, pA3G/F126-133, pA3G/F126-136, pA3G/F119-132, and pA3G/F126-132* were made by site-directed mutagenesis using the QuikChange II site-directed mutagenesis kit (Stratagene). In pA3G/F126-129, the amino acid sequence ¹²⁶FWDP¹²⁹ of A3G was mutated to YWER, the sequence found in A3F at the corresponding position. In pA3G/F126-132, the amino acid sequence ¹²⁶FWDPDYQ¹³² of A3G was mutated to the A3F sequence YWERDYR. In pA3G/F126-133, the amino acid sequence ¹²⁶FWDPDYQE¹³³ was mutated to YWERDYRR. In pA3G/F126-136, the amino acid sequence ¹²⁶FWDPDYQEALR¹³⁶ was mutated to YWERDYRRALC. In pA3G/F119-132, the amino acid sequence ¹¹⁹FVARLYYFWDPDYQ¹³² was mutated to SAARLYYYWERDYR. In pA3G/F126-132*, the amino acid sequence ¹²⁶FWDPDYQ¹³² was mutated to YWDRDYR. The alanine substitution mutants in WT A3G were also made by site-directed mutagenesis; where an

alanine was already present, it was substituted with an aspartic acid. In each case, the plasmid name indicates the amino acid position at which the changes were made; for example, A3G/133A134D had an alanine substitution at amino acid 133 and an aspartic acid substitution at position 134 of A3G. The structures of all plasmids were verified by DNA sequencing. The pcDNA-HVif (38) mutants DRMR>A4 and YRHHY>A5 were described previously (41); in DRMR>A4, amino acids 14 to 17 of Vif were substituted with four alanines; in YRHHY>A5, amino acids 40 to 44 of Vif were replaced with five alanines.

The modified human embryonic kidney cell line 293T (61) and the HeLa-derived HIV-1 reporter cell line TZM-bl (9, 56), which encodes the firefly luciferase gene under the control of the HIV-1 Tat-responsive promoter, were maintained in complete medium (CM), which consisted of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 1% penicillin-streptomycin, and 1% glutamine.

Virus production and titration. For virus production, 293T cells, seeded at 8×10^5 cells per well in six-well plates, were transfected using polyethylenimine (PEI) (Sigma), with modification of a previously described procedure (4). The DNA samples were diluted with 500 μ l of DMEM. A 500- μ l PEI (25 kDa)-DMEM solution was prepared (500 nM final concentration of PEI) and added to the DNA-DMEM samples dropwise while vortexing gently. The mixtures were incubated at room temperature for 20 min. CM was removed from the cells, and the DNA-PEI-DMEM samples were added to the plates. After 3 to 4 h, the DNA-PEI-DMEM was aspirated, and CM (1 ml) was added to the cells. The transfections were performed with the following plasmids: 3.33 μ g of the HIV-1 vector genome pHDV-EGFP (54); 0.67 μ g of vesicular stomatitis virus glycoprotein expression plasmid pHCMV-G (62); 0.67 μ g of either pcDNA-APO3G (21), which expresses A3G, pcDNA3.1-APOBEC3F (24), which expresses A3F, or the chimeric pFLAG-A3G-A3F constructs; and 4.5 μ g of either WT or mutant pcDNA-HVif. Where necessary, pcDNA3.1noMCS (41) was added to maintain equivalent amounts of DNA. The virus-containing supernatant was harvested 48 h after transfection, filtered through a 0.45- μ m filter, diluted in CM, and quantified using p24 CA enzyme-linked immunosorbent assay (Perkin-Elmer). TZM-bl cells were seeded at 4×10^3 cells per well in white flat-bottomed 96-well plates and infected with virus containing 5 ng of p24 CA 24 h later. Another 72 h later, the culture medium was removed and replaced with 100 μ l of CM without phenol red and 100 μ l of Britelite luciferase solution (Perkin-Elmer). After a 1-min incubation, the level of luciferase enzyme activity was measured using a LUMistar Galaxy luminometer.

Co-IP assays and immunoblotting analysis. 293T cells were seeded at 4×10^6 cells per 100-mm-diameter dish, and 24 h later, the cells transfected with 6 μ g of either pFLAG-A3G, pFLAG-A3F, or pFLAG-chimeric A3G-A3F; 9 μ g of either WT or mutant pcDNA-HVif; and 1.2 μ g pGL, which expresses green fluorescent protein from a cytomegalovirus immediate-early promoter (Invitrogen). Where necessary, pcDNA3.1noMCS was added to maintain equivalent amounts of DNA. The supernatant was removed 48 h posttransfection, and the cells were washed twice in 10 ml of phosphate-buffered saline. The cells were then lysed in 1 ml of lysis buffer (50 mM Tris-HCl [pH 7.4] with 150 mM NaCl, 1 mM EDTA, and 1% Triton X-100) containing protease inhibitor cocktail (Roche) by incubation with gentle agitation for 10 min. The cellular debris was removed by centrifugation at $10,000 \times g$ for 10 min, and the resulting supernatant was added to an anti-FLAG M2 agarose affinity gel (Sigma). The FLAG-tagged APOBEC3 proteins were allowed to bind to the agarose affinity gel by gentle rotation at 4°C overnight, after which any unbound proteins were removed by extensive washing with wash buffer (50 mM Tris-HCl [pH 7.4] with 150 mM NaCl). The bound protein complexes were then eluted by competition with a 3 $\times$ FLAG peptide (Sigma). The eluted complexes, as well as the input cell lysates, were then analyzed by polyacrylamide gel electrophoresis and Western blotting. The APOBEC3 proteins were detected using a rabbit anti-M2 polyclonal antibody (Sigma) at a 1:5,000 dilution, followed by a horseradish peroxidase-labeled goat anti-rabbit secondary antibody (Sigma) at a 1:10,000 dilution; the Vif proteins were detected using a rabbit anti-Vif polyclonal antibody (11) at a 1:5,000 dilution and the same secondary antibody at a 1:10,000 dilution. As a control for the amount of total protein, α -tubulin was detected using mouse anti- α -tubulin antibody (Sigma) at a 1:1,000 dilution, followed by a horseradish peroxidase-labeled goat anti-mouse secondary antibody at a 1:10,000 dilution. The proteins were visualized using the Western Lighting Chemiluminescence Reagent Plus kit (Perkin-Elmer).

Scintillation proximity assay. For determinations of cytidine deaminase activity, 293T cells seeded at 8×10^5 cells per well in six-well plates 1 day prior to transfection were transfected with 4 μ g of WT or alanine substitution mutant pcDNA-APO3G and either 1 μ g of pcDNA-HVif or 1 μ g of pcDNA3.1no MCS and 0.2 μ g of pGL (Invitrogen). The cells were harvested 48 h after transfection and lysed in 500 μ l of cell lysis buffer (20 mM Tris-HCl [pH 8.0] with 137 mM

NaCl, 2 mM EDTA, 1 mM NaVO₃, 10% glycerol, and 1% Triton X-100) containing Complete protease inhibitor cocktail (Roche), and the amount of total protein was determined using Bradford reagent (Bio-Rad). The level of cytidine deamination, and, hence, A3G activity, was determined using 0.2 µg of total cell protein using the scintillation proximity assay, as previously described (51).

RESULTS

Vif mutants capable of binding to only A3G or A3F are both able to bind to an A3G-A3F chimera. To determine the regions of A3G and A3F involved in binding to Vif, the previously described Vif mutants YRHHY>A5 and DRMR>A4 were used (Fig. 1A) (41). The DRMR>A4 mutant contains a four-alanine substitution of the A3F-binding domain (D¹⁴RMR¹⁷) and is able to bind only to A3G; therefore, it is termed the 3G binder. The YRHHY>A5 mutant contains a five-alanine substitution of the A3G-binding domain (Y⁴⁰RHHY⁴⁴) and is able to bind only to A3F; therefore, it is termed the 3F binder. These mutants were tested for their abilities to rescue a Vif-deficient HIV-1 vector from A3G, A3F, and an A3G-A3F chimera (13), which consists of the N terminus of A3G from amino acids 1 to 163 and the C terminus of A3F from amino acids 159 to 373 (G¹⁻¹⁶³F¹⁵⁹⁻³⁷³). As Fig. 1B shows, WT Vif and the 3G binder (but not the 3F binder) rescued the infectivity of viruses produced in the presence of A3G; the infectivity of viruses produced in the presence of A3F was rescued by WT Vif and the 3F binder (but not the 3G binder), as previously shown (41). In viral infectivity experiments, APOBEC3 activity was considered to be significant if viral infectivity was reduced more than twofold compared to that of the no-APOBEC3 control. Vif activity was considered to be significant if viral infectivity was increased more than twofold compared to that of the no-Vif control. Interestingly, the G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ chimera was inhibited by the 3G binder and the 3F binder as well as WT Vif, indicating that in A3G, the Vif-binding domain is located within amino acids 1 to 163, while in A3F, the Vif-binding domain is located within amino acids 159 to 373. To confirm this observation, we subcloned G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ into an N-terminally FLAG-tagged expression vector and performed co-IP studies with WT Vif, the 3G binder, and the 3F binder. As we had previously observed, A3G was able to efficiently coimmunoprecipitate WT Vif and the 3G binder but not the 3F binder; A3F was able to efficiently coimmunoprecipitate WT Vif and the 3F binder but not the A3G binder (Fig. 1C, lanes 1 to 6). In contrast, the chimera FLAG-G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ was able to coimmunoprecipitate WT Vif as well as both the 3G binder and the 3F binder (Fig. 1C, lanes 7, 8, and 9). The co-IP analysis was consistent with the observations that both the 3G binder and the 3F binder suppressed the ability of the G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ chimera to block HIV-1 replication (Fig. 1B). Furthermore, the results demonstrated that amino acids 1 to 163 of A3G contain a Vif-binding domain, whereas in A3F, the Vif-binding domain is located between amino acids 159 and 373. A Vif-induced degradation of APOBEC3 proteins was not observed in the cell lysates, because the APOBEC3- and Vif-expressing plasmids were cotransfected at a 1:1.5 molar ratio, allowing efficient co-IP and immunodetection of the APOBEC3 proteins in the cell lysates. We previously reported that when APOBEC3- and Vif-expressing plasmids were cotransfected at a molar ratio of 1:5, the degradation of both A3G and A3F was

FIG. 1. The Vif interaction domain is located in the N terminus of A3G and the C terminus of A3F. (A) Schematic representation of Vif mutants defective in A3G and A3F binding. The top line depicts the first 50 amino acids of WT Vif, the second line shows the four alanine substitutions made in the 3G binder (DRMR>A4), and the third line shows the five alanine substitutions made in the 3F binder (YRHHY>A5). Dashes indicate identical amino acids. (B) Effect of A3G, A3F, and the G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ chimera on HIV-1 infectivity in the presence and absence of Vif. To determine APOBEC3 sensitivity to WT and mutant Vif proteins, 293T cells were transfected with the HIV-1 vector pHDV-EGFP, a vesicular stomatitis virus glycoprotein expression plasmid (pHCMV-G), an APOBEC3-expressing plasmid, and a Vif-expressing plasmid (WT Vif, the 3G binder, or the 3F binder). HIV-1-derived vector-enhanced green fluorescent protein (EGFP) virus was also prepared in the presence of the APOBEC3 proteins but in the absence of any Vif proteins to determine the function of the APOBEC3 proteins. As a positive control, HDV-EGFP virus was prepared in the absence of any APOBEC3 proteins. The infectivity of the virus produced from the transfected cells harvested after 48 h was determined by infection of TZM-bl indicator cells and quantitation of the resulting luciferase enzyme activity. The data shown are plotted as the infectivity relative to that produced in the absence of any APOBEC3 proteins (not shown), which was set to 100%, with standard errors of the means (SEM) from three independent experiments. (C) Co-IP assays to determine binding of Vif and APOBEC3 proteins. To assess the level of binding between the APOBEC3 proteins and the WT and mutant Vif proteins, 293T cells were cotransfected with N-terminally FLAG-tagged versions of A3G, A3F, or G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ along with WT Vif, the 3G binder, or the 3F binder. The transfected cell lysates were analyzed for the expression of the APOBEC3 and Vif proteins using anti-FLAG and anti-Vif antibodies, respectively, by Western blotting. The cell lysates were also analyzed for α-tubulin to control for the amount of cell lysate examined. Additionally, the cell lysates were analyzed in an immunoprecipitation assay using anti-FLAG resin to immunoprecipitate the FLAG-tagged APOBEC3 proteins. The immunoprecipitated proteins were then analyzed by Western blotting using anti-FLAG and anti-Vif antibodies. A representative analysis is shown.

readily apparent in the presence of WT Vif, whereas the 3G binder induced the degradation of only A3G and the 3F binder induced the degradation of only A3F (41).

To identify the locations of these Vif-binding domains more precisely, we constructed a series of N-terminally FLAG-tagged chimeric proteins with either the N terminus of A3G and the C terminus of A3F (GF chimeras) (Fig. 2A and B) or the N terminus of A3F and the C terminus of A3G (FG chimeras) (Fig. 2A and C). In addition, we constructed A3G/F126-129, A3G/F126-132, and A3G/F126-132* chimeras in which the N and C termini were derived from A3G, while a portion of the central region downstream of the first catalytic domain (catalytic domain 1 [CD1]) was derived from A3F (Fig. 2B). We also constructed an A3F/G291/F chimera in which the N and C termini were derived from A3F, while the region downstream of the second catalytic domain (CD2) was derived from A3G (Fig. 2C). The names of the chimeras indicate the amino acid sequences derived from either A3G or A3F; for example, $G^{1-99}F^{99-373}$ contains amino acids 1 to 99 of A3G followed by amino acids 99 to 373 of A3F; the crossover points are shown in detail in Fig. 2A. To rule out the possibility of false-negative or false-positive results due to the misfolding and/or mislocalization of the proteins, each chimera was first tested for its ability to inhibit the replication of a Vif-deficient HIV-1 vector, and only those chimeras with antiviral activity were used for further study (Fig. 2B and C). This criterion led to the exclusion of a number of chimeras that failed to inhibit HIV-1 replication because they either expressed aberrant proteins or were poorly expressed (data not shown).

A3G-A3F chimeric proteins are expressed and are targeted for degradation by Vif. We sought to determine the steady-state levels of expression of the A3G-A3F chimeric proteins and their sensitivity to WT Vif. We cotransfected the GF chimeras and WT Vif expression plasmids at a 1:5 molar ratio and determined the levels of the chimeric proteins and Vif proteins present in cell lysates (Fig. 3A). The results showed that when a fivefold molar excess of the Vif expression plasmid was cotransfected, WT A3G was efficiently degraded, and little or no A3G could be detected by Western blotting. The results also showed that the GF chimeras were also efficiently degraded in the presence of Vif, indicating that these proteins interacted with Vif and were targeted for proteasomal degradation. The steady-state amount of the $G^{1-321}F^{315-373}$ protein was significantly less than that of the other GF chimeras, indicating that it was either expressed poorly or more efficiently degraded than WT A3G or other GF chimeras. Despite its lower steady-state level, the $G^{1-321}F^{315-373}$ protein was expressed at sufficiently high levels in the infectivity assays to efficiently inhibit HIV-1 replication. In contrast to the GF chimeras, the A3G/F126-129, A3G/126-132, and A3G/126-132* chimeric proteins were not efficiently degraded in the presence of Vif, indicating that these chimeras were Vif resistant.

We also determined the steady-state levels and Vif sensitivity of the FG chimeric proteins (Fig. 3B). At a 1:10 molar ratio of A3F to Vif, the WT A3F protein was efficiently degraded. The FG chimeras $F^{1-248}G^{257-384}$ and $F^{1-282}G^{291-384}$ were resistant to degradation, whereas the FG chimeras $F^{1-300}G^{309-384}$ and $F^{1-314}G^{322-384}$ were sensitive to Vif-mediated degradation. The A3F/G291/F chimera was expressed at a lower steady-

state level but was resistant to Vif-mediated degradation; despite the lower steady-state levels of expression, this chimeric protein was expressed at sufficiently high levels to inhibit HIV-1 replication under the conditions of our infectivity assays.

Amino acids 121 to 149 of A3G are essential for binding to the YRHYY region of Vif but not sufficient for A3G degradation. To define the region of A3G involved in binding to the YRHYY region of Vif further, the GF chimeras $G^{1-99}F^{99-373}$, $G^{1-120}F^{120-373}$, $G^{1-149}F^{145-373}$, $G^{1-163}F^{159-373}$, and $G^{1-321}F^{315-373}$ were assessed for their abilities to bind to WT Vif, the 3G binder, and the 3F binder by co-IP assays. WT A3G and WT Vif were included as positive and negative controls, respectively. The chimera $G^{1-99}F^{99-373}$ bound to WT Vif and the 3F binder but not to the 3G binder (Fig. 4A, lanes 4 to 6), whereas WT A3G bound to WT Vif and the 3G binder but not the 3F binder (Fig. 4A, lanes 1 to 3). This difference in co-IP indicated that the A3G portion of $G^{1-99}F^{99-373}$ (amino acids 1 to 99) is insufficient for binding to the YRHYY region of Vif but that the A3F portion from amino acids 99 to 373 contains a domain that interacts with WT Vif and the 3F binder (Fig. 4A, lanes 4 and 6). Presumably, the binding of WT Vif to the $G^{1-99}F^{99-373}$ chimera occurred through its A3F-binding DRMR region and not its A3G-binding YRHYY region, since the 3G binder was incapable of binding to $G^{1-99}F^{99-373}$. The same phenotype was seen with the GF chimera $G^{1-120}F^{120-373}$ (Fig. 3A, lanes 7 to 9), indicating that amino acids 1 to 120 of A3G do not contain a YRHYY-binding domain but that amino acids 120 to 373 of A3F still contain a DRMR-binding domain. Binding of the 3G binder was seen only when a larger section of A3G, from amino acids 1 to 149 ($G^{1-149}F^{145-373}$) (Fig. 4A, lane 11) or amino acids 1 to 163 ($G^{1-163}F^{159-373}$) (Fig. 1C and 4A, lane 14), was present within the chimera. Unlike the previous two chimeras, $G^{1-149}F^{145-373}$ and $G^{1-163}F^{159-373}$ bound to WT Vif and both the 3G and 3F binders (Fig. 4A, lanes 10 to 15), indicating that both A3G YRHYY and A3F DRMR interaction domains were present. The final GF chimera, $G^{1-321}F^{315-373}$, had a Vif-binding pattern identical to that of WT A3G in that it bound to WT Vif and the 3G binder but not the 3F binder (Fig. 4A, lanes 16 to 18). Furthermore, its inability to bind to the 3F binder demonstrated that amino acids 315 to 373 of A3F were not sufficient for binding to the DRMR region of Vif. Based on the comparison of $G^{1-120}F^{120-373}$ and $G^{1-149}F^{145-373}$ (Fig. 4A, lanes 8 and 11), and consistent with the results obtained with the other GF chimeras, we conclude that the A3G domain that binds to the YRHYY region of Vif is located between amino acids 121 and 149.

We next sought to determine whether the ability of the GF chimeras to bind to Vif resulted in the blocking of their antiviral activity. To achieve this, the GF chimeras were tested in an HIV-1 infectivity assay for their sensitivity to WT Vif, the 3G binder, and the 3F binder. The Vif sensitivity profiles (Fig. 4B) were then compared with the binding profiles (Fig. 4A) to determine binding functionality. As controls, WT A3G and WT A3F were also tested and showed the expected Vif sensitivities (Fig. 4B). The chimera $G^{1-321}F^{315-373}$ had a Vif sensitivity profile identical to that of A3G; this chimera was sensitive to WT Vif and the 3G binder, indicating that the first 321 amino acids of A3G contain a functional YRHYY-binding domain (Fig. 4B). Additionally, the $G^{1-321}F^{315-373}$ chimera was

FIG. 2. Inhibition of HIV-1 Δ Vif by the A3G/A3F chimeras. (A) Schematic representation of the crossover points of each chimera. Alignments of the A3G and A3F amino acid sequences and the crossover points in each chimera are shown. Identical amino acids in A3G and A3F at the crossover junctions are shown in boxes; dots indicate gaps in the sequence. (B) Effects of the GF and A3G/F126-129, A3G/F126-132, and A3G/F126-132* chimeras on HIV-1 infectivity. A3G is depicted in black, and A3G CD1 and CD2 are depicted in dark gray. A3F is depicted in light gray, and the A3F CD1 and CD2 are depicted in white. To determine GF chimera function, 293T cells were transfected with pHDV-EGFP, pHCMV-G, and an APOBEC3-expressing plasmid. As a positive control, HDV-EGFP virus was prepared in the absence of any APOBEC3 proteins. After 48 h, the infectivity of the virus produced from the transfected cells was determined by infection of TZM-bl indicator cells and quantitation of the resulting luciferase enzyme activity. The data shown are plotted as the infectivity relative to that produced in the absence of any APOBEC3 proteins, which was set to 100%, with SEM from three independent experiments. (C) Effects of the FG and A3F/G291/F chimeras on HIV-1 infectivity. The color scheme and experimental protocol is the same as that described above.

FIG. 3. Expression levels and Vif sensitivity of A3G-A3F chimeras. (A) Expression of A3G-A3F chimeras in the absence or presence of Vif. The GF chimera-expressing plasmids and WT Vif expression plasmids were cotransfected into 293T cells. An APOBEC3-to-Vif expression plasmid molar ratio of 1:5 was used for the cotransfections. The transfected cell lysates were analyzed by Western blotting for the expression of the APOBEC3 and Vif proteins using anti-FLAG and anti-Vif antibodies, respectively. The cell lysates were also analyzed for tubulin expression using an anti-tubulin antibody to control for the amount of cell lysate examined. (B) Expression of A3F-A3G chimeras in the presence and absence of Vif. The FG chimera-expressing plasmids and WT Vif-expressing plasmid were cotransfected into 293T cells. An APOBEC3-to-Vif molar ratio of 1:10 was used for the cotransfections. The APOBEC3, Vif, and tubulin proteins were detected by Western blotting as described in the text.

insensitive to the 3F binder, as predicted based on the fact that it did not bind to the 3F binder (Fig. 4A, lane 18). The GF chimeras $G^{1-99}F^{99-373}$ and $G^{1-120}F^{120-373}$ were sensitive to WT Vif and the 3F binder, demonstrating that binding to the A3F portion of these chimeras was functional (Fig. 4B). As expected, neither $G^{1-99}F^{99-373}$ nor $G^{1-120}F^{120-373}$ was sensitive to the 3G binder due to their above-described inability to interact with it (Fig. 4A, lanes 5 and 8). Intriguingly, the chimera $G^{1-149}F^{145-373}$ was resistant to the 3G binder despite binding to it (Fig. 4A, lane 11). These results are consistent with previous observations by us and others that binding to Vif is necessary but not sufficient for degradation (41, 60, 65). Thus, amino acids 121 to 149 of A3G are sufficient for binding to the YRHHY region of Vif but not for A3G degradation, which requires a larger section from amino acids 121 to 163 ($G^{1-163}F^{159-373}$) (Fig. 1C and 4B).

Amino acids 126 to 132 of A3G are critical for binding to the YRHHY region of Vif. To define the Vif-binding region of A3G further, additional mutants in which 2 to 4 amino acids within the Vif-binding domain (amino acids 121 to 149) were substituted with alanines were constructed; if an alanine was already

FIG. 4. The A3G Vif-binding domain is localized to amino acids 121 to 149. (A) Co-IP assays to determine binding of Vif and GF chimeras. Binding of the GF chimeras to WT Vif, the 3G binder, and the 3F binder was determined as described in the legend of Fig. 1C by co-IP and Western blotting. A representative analysis is shown. (B) Effects of GF chimeras on HIV-1 infectivity and their sensitivity to Vif proteins. HIV-1 infections of TZM-bl cells were carried out as described in the legend of Fig. 1B. The SEM determined from two to four independent experiments are shown.

present, it was substituted with an aspartic acid (Fig. 5A). Analysis of the sensitivity of these mutants to Vif using the scintillation proximity assay (51) for A3G enzymatic activity in transfected cells showed that amino acids 133 to 149 were not involved in Vif binding; all of the mutants remained sensitive to Vif-induced degradation, as measured by the loss of cytidine deaminase activity in the presence of Vif (Fig. 5B). Unfortunately, cells transfected with alanine mutants of amino acids 121 to 132 did not have measurable levels of cytidine deaminase activity, making further definition of the Vif-binding region impossible using this approach. We therefore constructed a series of chimeras in which amino acids 126 to 132 of A3G were progressively replaced with those present at equivalent positions in A3F (amino acids 121 to 125 are identical in A3G and A3F) (Fig. 2A). In the A3G/F126-129 chimera, F126Y, D128E, and P129R substitutions were introduced, while in the A3G/F126-132 chimera, F126Y, D128E, P129R, and Q132R substitutions were generated, and in the A3G/F126-132* chimera, F126Y, P129R, and Q132R substitutions were made (Fig. 5A). The chimeras A3G/F126-133, A3G/F126-136, and

FIG. 5. Amino acids 126 to 132 of A3G are critical for binding to Vif. (A) Schematic representation of mutants of the Vif-binding domain of A3G. The top line depicts amino acids 121 to 150 of WT A3G, the next seven lines depict the alanine and aspartic acid substitution mutants, and the last three lines depict the A3G/F126-129, A3G/F126-132, and A3G/F126-132* chimeras; dashes indicate identical amino acids. (B) Effects of alanine substitution mutations on cytidine deaminase activity. The alanine substitution mutants shown in A were transfected into 293T cells in the presence or absence of Vif, the cell lysates were harvested 48 h posttransfection, and cytidine deaminase activity was determined using 0.2 μ g of total protein. The data shown are plotted as the level of A3G activity relative to that obtained from WT A3G in the absence of any Vif, which was set to 100%. The SEM determined for two to three independent experiments are shown. (C) Effects of A3G/F chimeras on HIV-1 infectivity in the presence or absence of Vif. The chimeras A3G/F126-129, A3G/F126-132, and A3G/F126-132* were tested for their sensitivity to WT Vif and both the 3G and 3F binders as described in the legend of Fig. 1B. The SEM determined for two to four independent experiments are shown. (D) Co-IP assays to determine binding of Vif and A3G/F chimeras. The binding of the A3G/F126-129, A3G/F126-132, and A3G/F126-132* chimeras to the 3G binder and the 3F binder was determined as described in the legend of Fig. 1C by co-IP and Western blotting. A representative analysis is shown.

A3G/F119-132 had larger sections from amino acids 119 to 136 of A3G replaced; however, these chimeras lacked antiviral activity and, as such, were not studied further (data not shown). The chimeras A3G/F126-129, A3G/F126-132, and A3G/F126-132* (Fig. 5A) were able to block HIV-1 replication even in the presence of WT Vif, the 3G binder, and the 3F binder (Fig. 5C), indicating that they either did not bind to Vif or were not degraded upon Vif binding as previously reported for the D128K A3G mutant (41, 60).

To determine whether the A3G/F126-129, A3G/F126-132, and A3G/F126-132* chimeras were Vif resistant because of a lack of Vif binding or a lack of degradation, we characterized the ability of these chimeras to bind to WT Vif, the 3G binder, and the 3F binder in co-IP assays (Fig. 5D and data not shown). Consistent with their insensitivity to the 3F binder, the A3G/F126-129, A3G/F126-132, and A3G/F126-132* chimeras were unable to bind to the 3F binder (Fig. 5D, lanes 5, 7, and 9). All chimeras exhibited reduced binding to both WT Vif (data not shown) and the 3G binder (Fig. 5D, compare lane 2

with lanes 4, 6, and 8), consistent with the idea that amino acids located between 126 and 132 are involved in binding; the substitution of amino acids 126, 128, 129, and 132 in A3G/F126-132 resulted in a severe reduction in Vif binding; this reduction in binding was also observed with the substitution of amino acids 126, 129, and 132 in A3G/F126-132*, suggesting that the substitution of D128 with E was not critical for the loss of Vif binding. The observation that Vif binding was not completely eliminated with these substitutions suggests that other amino acids in A3G may also contribute to Vif binding.

Amino acids 283 to 300 of A3F are critical for binding to the DRMR region of Vif and for A3F degradation. A comparison of the G¹⁻¹⁶³F¹⁵⁹⁻³⁷³ and the G¹⁻³²¹F³¹⁵⁻³⁷³ chimeras (Fig. 4A and B) indicated that the DRMR-binding determinant in A3F was located between amino acids 159 and 315, as the 3F binder could bind to the former but not the latter chimera. Further attempts to narrow down this region using GF chimeras with crossover points surrounding CD2 proved unsuccessful, as these chimeras were nonfunctional (data not shown). There-

fore, to define the region of A3F involved in binding to the DRMR region of Vif further, a series of FG chimeras were constructed and shown to retain their antiviral activities (Fig. 2C). These chimeras, $F^{1-248}G^{257-384}$, $F^{1-282}G^{291-384}$, $F^{1-300}G^{309-384}$, and $F^{1-314}G^{322-384}$, were assessed for their abilities to bind to the 3G binder and the 3F binder in co-IP assays (Fig. 6A). WT A3F and WT Vif were included as positive and negative controls, respectively. The chimeras $F^{1-300}G^{309-384}$ and $F^{1-314}G^{322-384}$ showed binding profiles that matched those seen with A3F (Fig. 6A, compare lanes 8 to 11 with lanes 2 and 3), indicating that they contained a DRMR-A3F interaction domain but not a YRHYY-A3G interaction domain; these results were consistent with results of binding studies performed with the GF chimeras. The chimeras $F^{1-248}G^{257-384}$ and $F^{1-282}G^{291-384}$ lost binding to the 3F binder (Fig. 5A, lanes 5 and 7); thus, a comparison of these two vectors with $F^{1-300}G^{309-384}$, which retained binding to the 3F binder, indicated that the DRMR-A3F binding determinant is located between amino acids 283 and 300 of A3F. We also observed that the $F^{1-248}G^{257-384}$ and $F^{1-282}G^{291-384}$ chimeras were able to bind to WT Vif (at a lower efficiency than that of WT A3F), even though they were unable to bind to the 3F binder (data not shown). This observation suggests that the C-terminal end of A3G, or the N-terminal half of A3F, has a determinant that also interacts with Vif but that this determinant is not accessible for Vif binding in WT APOBEC3 proteins; it should be noted that this determinant does not seem to be involved in a functional interaction that leads to the degradation of the APOBEC3 proteins.

We next assessed whether the DRMR-A3F interaction involving A3F amino acids 283 to 300 was capable of inducing the degradation of A3F by using the HIV-1 infectivity assay. As described above, the Vif sensitivity profiles (Fig. 6B) were compared with the binding profiles (Fig. 6A) to determine the functionality of binding. WT A3G and WT A3F were tested and showed the expected Vif sensitivities (Fig. 6B). The chimera $F^{1-300}G^{309-384}$ was sensitive to WT Vif and the 3F binder but resistant to the 3G binder (Fig. 6B). These data are consistent with the binding data and indicate that amino acids 283 to 300 of A3F are critical for both binding and degradation. Surprisingly, the chimera $F^{1-314}G^{322-384}$ appeared to be resistant to WT Vif and the 3F binder despite binding to both of them (Fig. 6A and B and data not shown). The $F^{1-314}G^{322-384}$ chimera is fourfold more efficient than WT A3F at inhibiting HIV-1 replication; we hypothesize that because of the higher potency of this chimera, the ability of Vif to rescue viral replication was not detectable. The chimeras $F^{1-248}G^{257-384}$ and $F^{1-282}G^{291-384}$ were also resistant to all Vif proteins. Again, this was expected for the 3G binder and the 3F binder, as they did not interact in the co-IP assay. Furthermore, it suggests that the interaction between these chimeras and WT Vif (data not shown) is nonfunctional.

To confirm the involvement of amino acids 283 to 300 of A3F in Vif binding, the chimera A3F/G291/F was constructed and shown to be functional (Fig. 2C). This chimera consisted of amino acids 1 to 282 of A3F, followed by amino acids 291 to 319 of A3G and amino acids 315 to 373 of A3F at the C terminus. Thus, based on our previous observations, the A3F/G291/F chimera should not contain an A3F Vif-binding domain or an A3G Vif-binding domain. As expected, the A3F/

FIG. 6. Amino acids 283 to 300 of A3F are critical for binding to Vif. (A) Co-IP assays to determine binding of Vif to the FG chimeras. The binding of the FG chimeras to the 3G binder and the 3F binder was determined as described in the legend of Fig. 1C by co-IP and Western blotting. A representative analysis is shown. (B) Effects of the FG and A3F/G291/F chimeras on HIV-1 infectivity and their sensitivity to Vif proteins. Determination of the Vif sensitivity of the FG and A3F/G291/F chimeras was carried out as described in the legend of Fig. 1B. The SEM determined for two to four independent experiments are shown. (C) Co-IP assays to determine binding of Vif to the A3F/G291/F chimera. The binding of the A3F/G291/F chimera to WT Vif, the 3G binder, and the 3F binder was determined as described in the legend of Fig. 1C by co-IP and Western blotting. A representative analysis is shown.

G291/F chimera did not bind to any of the Vif proteins efficiently, unlike A3F, which bound strongly to both WT Vif and the 3F binder (Fig. 6C). Furthermore, in the infectivity assay, the A3F/G291/F chimera was resistant to all Vif proteins

FIG. 7. Sequence and model structure of Vif binding domains of A3G and A3F. (A) Alignment of A3G and A3F sequences within the A3G Vif- and A3F Vif-binding sites. Sequence alignment of the A3G Vif-binding domain with the corresponding region in A3F and the A3F Vif-binding domain with the corresponding region in A3G. Dashes indicate identical amino acids, and dots indicate gaps in the sequence. The residues in boldface type are those previously shown to be critical for Vif binding. (B) Structure of the C-terminal domain of A3G and location of the N-terminal Vif-binding domain mapped onto the corresponding region (shown in green). The highlighted residues span amino acids 315 to 322 of A3G, which correspond to amino acids 126 to 132 of A3G. The location in the C terminus of the A3F Vif-binding domain is shown within the boxed area. β -Sheets 1 to 5 are shown in pink, and α -helices 1 to 5 are shown in yellow. (C) Structure of the C-terminal domain of A3G and location of the Vif-binding domain of

(Fig. 6B), thereby confirming the role of this region of A3F in binding to Vif.

DISCUSSION

Since the discovery of A3G (45) and other APOBEC3 proteins, numerous studies have demonstrated that these proteins constitute a powerful antiviral defense mechanism. In order to overcome this defense mechanism, HIV-1 has acquired the ability to express Vif, which interacts with the APOBEC3 proteins and induces their degradation. It has now become evident that the Vif-APOBEC3 interaction is essential for viral replication, thus making it an attractive target for antiviral drug development. However, the specific regions of the APOBEC3 and Vif proteins that interact have been the focus of only a limited number of studies. Initial studies indicated that the substitution of the D128 residue of A3G with lysine generated a Vif-resistant version of the protein, and while some studies indicated that the D128K mutant did not bind to Vif (3, 31, 44), our results indicated that it was Vif resistant despite an ability to bind to Vif (41, 60). Another study used fragments of A3G to map the Vif-binding domain to amino acids 54 to 124 of A3G (6). However, recently, a similar approach mapped the Vif-binding domain to a different region between amino acids 105 and 156 (65). In addition, Huthoff and Malim carried out substitution mutation analysis of amino acids 124 to 132 and found that amino acids 128 to 130 were essential for the Vif interaction (19). Importantly, the region of A3F involved in Vif binding had not previously been studied.

We recently showed that the D¹⁴RMR¹⁷ and Y⁴⁰RHHY⁴⁴ regions of Vif play a critical role in binding to A3F and A3G, respectively (41). Using previously identified Vif mutants that bind only to A3G or A3F (41), along with a series of A3G/A3F chimeras, we have now identified the A3F domain that interacts with the Vif DRMR region and addressed the disparity in data from previous publications regarding the Vif-binding domain of A3G. The results of these studies revealed, for the first time, that the domains of A3G and A3F that interact with the YRHHY and DRMR regions of Vif, respectively, are located in distinct regions within the two proteins. Amino acids 121 to 149 of A3G were sufficient for binding to the YRHHY region of Vif, and mutational analysis further localized the YRHHY binding region to amino acids 126 to 132 (Fig. 7A) within the domain identified previously by Zhang et al. and overlapping the region reported previously by Huthoff and Malim (19, 65). The substitution of the amino acids in A3G with the corresponding amino acids in A3F abrogated binding to the YRHHY region of Vif, further confirming the role of this region in A3G Vif binding. Within the A3G domain, it is unclear which specific amino acid differences between A3G and A3F are responsible for the loss of binding to the YRHHY

A3F mapped onto the corresponding region (shown in green). The highlighted residues span amino acids 292 to 308 of the C terminus of A3G, which correspond to amino acids 283 to 300 of A3F. The location of the N-terminal A3G Vif-binding domain is shown within the boxed area. β -Sheets 1 to 5 are shown in pink, and α -helices 1 to 5 are shown in yellow.

region of Vif. In accordance with our previous findings (41, 60), D128 appeared to not be the sole determinant of binding, as A3G/126-132, which contains a D128E substitution, showed a reduction in binding similar to that of A3G/126-132*, which retained the D128 residue. These observations suggest that no one amino acid is acting as the binding determinant; instead, multiple amino acids collectively contribute to the interaction. A recent study by Gooch and Cullen also demonstrated the importance of this region in the interaction between the APOBEC3 and Vif proteins (12).

Although amino acids 126 to 132 of A3G were sufficient for binding to the YRHHY region of Vif, they were not sufficient for Vif-induced degradation; instead, amino acids 126 to 163 of A3G were required for degradation. These data are in agreement with, and further define, data from previous studies indicating that amino acids 105 to 245 of A3G were required for functional binding (65). These observations suggest a number of hypotheses. Firstly, it is possible that following binding at the primary binding site, a secondary binding occurs between the APOBEC3 proteins and Vif or the APOBEC3 proteins and the cellular proteins involved in the degradation process. Secondly, the additional sequences from A3G may be required for a specific conformational change in A3G to occur upon Vif binding that is needed to induce A3G degradation. Alternatively, the lysine residues present in the additional sequence may be required for polyubiquitination prior to degradation (17). However, Dang et al. recently reported that the removal of all the lysine residues of A3G has no effect on the Vif-induced degradation of A3G (7). Further studies will be required to determine the role of the additional sequences in A3G degradation. A recent study suggested that while amino acids 1 to 196 of A3G are sufficient for binding to Vif, they are not sufficient for Vif-induced degradation (12). The apparent discrepancy between those data and ours may be due to differences between their use of truncation mutants and our functional chimeric APOBEC3 proteins.

In agreement with our initial hypothesis that distinct determinants of A3G and A3F are involved in Vif binding, the A3F region that binds to the DRMR region did not map to the same region as the YRHHY-binding domain of A3G and instead was localized to the C terminus of A3F between amino acids 283 and 300. Interestingly, despite the high degree of homology between the A3G YRHHY-binding domain and the corresponding region of A3F, little similarity between the A3F DRMR-binding domain and the corresponding region of A3G exists (Fig. 7A). The fact that Vif evolved a distinct domain to interact with A3F and target it for degradation indicates that A3F is an important host restriction factor that HIV-1 must overcome in order to replicate efficiently. It is possible that A3F and A3G act in different cell types (activated CD4⁺ T cells, macrophages, and dendritic cells) to inhibit HIV-1 replication.

The data presented here are the first to define the domain of A3F that interacts with the DRMR region of Vif and show that the A3G and A3F domains are distinct; however, the ultimate goal would be structural comparisons between A3G and A3F, preferably in the context of Vif. In the absence of such data, we have mapped the Vif-interacting regions onto the recently determined solution structure of the C-terminal domain of A3G (5). Using the homology between the N-terminal and C-termi-

nal catalytic domains of A3G, we have mapped the A3G YRHHY-binding site (Fig. 7B). Similarly, we have used the structure to locate the A3F DRMR-binding site C terminal to the catalytic domain (Fig. 7C). The A3G YRHHY-binding site is located on a loop between α -helix 3 and β -sheet 4, whereas the A3F DRMR-binding site is located on part of β -sheet 4, a loop, and part of α -helix 2. It is interesting to speculate that the two loops separated by β -sheet 4 may be critical for both A3G and A3F interactions with Vif albeit within different regions of the proteins.

Analysis of the A3G/A3F chimeras also revealed that the F¹⁻³¹⁴G³²²⁻³⁸⁴ chimera was much more active and more Vif resistant than WT A3F. This interesting observation suggests that the C-terminal end of A3F restrains the cytidine deaminase activity and antiviral activity of A3F. In recent studies, it was found that human APOBEC3H has been independently inactivated on at least two occasions in the human lineage (39). This observation has led to the idea that antiviral defense using cytidine deaminases comes at a mutagenic cost to the host (20). Presumably, the host must achieve an optimal balance between achieving the necessary level of antiviral activity and minimizing mutagenic activity that damages its own genome. The fact that these chimeras are more Vif resistant, despite binding to Vif, suggests that secondary conformational changes that occur upon binding to Vif are affected by the chimeric protein structure. Further studies of this and other A3G/A3F chimeras could lead to additional interesting insights into the structure and function of the APOBEC3 proteins.

These studies, taken together with our previous data defining the A3G- and A3F-binding domains of Vif, should facilitate the design of specific small-molecule inhibitors of the interaction, thereby allowing the host innate immune system to target HIV-1. The fact that different regions of A3G and A3F interact with different domains on Vif suggests that inhibitors of one of the interactions are unlikely to interfere with the other interaction. Thus, it might be possible to combine inhibitors of both interactions to attain more effective antiviral strategies.

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