A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor

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The HIV type 1 (HIV-1) virion infectivity factor (Vif) protein blocks the action of the host defense factor APOBEC3G in human cells, thereby allowing release of infectious virions, but fails to inhibit similar APOBEC3G proteins present in some simian cells. Conversely, the Vif protein encoded by the African green monkey (agm) simian immunodeficiency virus (SIV) can block agm APOBEC3G function but fails to inhibit human APOBEC3G. This difference plays a key role in determining the primate species tropism of HIV-1 and SIV agm. Here, we demonstrate that a single APOBEC3G residue, which is an aspartic acid in human APOBEC3G and a lysine in agm APOBEC3G, controls the ability of the HIV-1 Vif protein to bind and inactivate these host defense factors. These data identify a critical charged residue that plays a key role in mediating the formation of the distinct Vif:APOBEC3G complexes formed in human and simian cells. Moreover, these results suggest that the biological barrier preventing the entry of additional SIV into the human population as zoonotic infections is potentially quite fragile.

The HIV type 1 (HIV-1) virion infectivity factor (*vif*) gene product is essential for virus replication in primary human cells in culture (1, 2). In the absence of a functional Vif protein, HIV-1 virions are produced in normal amounts but are unable to initiate productive infections (3–5). The *vif* gene is conserved in all known simian immunodeficiency viruses (SIVs) and is also required for SIV replication in primary cells and for SIV-induced pathogenesis *in vivo* (6).

Efforts to understand the mechanisms of action of this essential viral gene product led to the demonstration that Vif functions by blocking the activity of a cellular antiretroviral defense protein termed APOBEC3G (7). In the absence of Vif, human APOBEC3G (h3G) is specifically packaged into HIV-1 virions and then acts in newly infected cells to disrupt provirus formation by extensively editing dC residues to dU on the DNA minus strand during reverse transcription (8–11). Vif reverses this inhibition by specifically binding to h3G in virus producer cells and then blocking h3G virion incorporation directly and/or targeting h3G for ubiquitination and degradation by the proteasome (10, 12–18).

An important attribute of lentiviral Vif proteins is that their function is generally highly species-specific. Thus, HIV-1 Vif is unable to prevent inhibition of HIV-1 replication not only by rodent APOBEC3G orthologs but also by the APOBEC3G proteins encoded by African green monkeys (agm), Sykes monkeys, and rhesus macaques (10, 19). Similarly, the Vif protein encoded by SIVagm is active against the agm APOBEC3G (agm3G) protein but does not inhibit h3G. These data have led to the hypothesis that Vif may be a key regulator of the primate species tropism of primate immunodeficiency viruses (10, 19). Thus, the chimpanzee variant of SIV, which gave rise to HIV-1, and sooty mangabey SIV, which gave rise to HIV-2, encode Vif proteins that are able to block h3G function, thereby permitting virus replication in human cells (10). In contrast, highly prevalent SIVs that encode Vif proteins unable to inhibit h3G, such as SIVagm (19), replicate poorly in primary human cells (20) and, perhaps as a result, have been unable to cross over into the human population as zoonotic infections.

Here, we have sought to define the molecular basis for the primate species tropism of HIV-1 and SIVagm Vif. We demonstrate that each Vif protein is able to specifically bind and inhibit the APOBEC3G protein from its own species but not from the heterologous species. Remarkably, this discrimination maps to a single residue in the ≈384-aa APOBEC3G protein, which is an aspartic acid residue in h3G and a lysine in agm3G. This surprising result suggests that the marked species tropism of primate lentiviral Vif proteins, and possibly of primate lentiviruses in general, may reflect quite minor differences in the sequence of the target APOBEC3G protein.

Methods

Molecular Clones. An h3G cDNA was amplified by PCR from human leukocyte Quick-clone cDNA (BD Biosciences, Clontech). The PCR product (*Kpn*I/*Eco*RI fragment) was then subcloned in frame into a pcDNA3-based expression plasmid, termed pcDNA3-HA, that had been modified to encode a C-terminal triple influenza hemagglutinin (HA) epitope tag, to give ph3G-HA. Several agm3G cDNAs were cloned from the agm kidney cell line CV-1. Briefly, poly(A)$^+$ mRNA was isolated and a cDNA library constructed by using the ZAP cDNA synthesis kit, the Uni-ZAP XR vector, and a Gigapack III Gold packaging kit (Stratagene). The CV-1 cDNA library was then probed with a radiolabeled h3G cDNA fragment by using low-stringency hybridization. After plaque purification, several independent but identical full-length agm3G clones were identified and sequenced (Fig. 6, which is published as supporting information on the PNAS web site). After PCR amplification, the agm3G cDNA was cloned in-frame into pcDNA3-HA, as described above, to give pagm3G-HA.

Expression plasmids encoding HA epitope-tagged h3G/agm3G chimeras and point mutants were constructed from ph3G-HA or pagm3G-HA either by using shared unique restriction sites or by recombinant PCR. All mutations were verified by DNA sequence analysis. Expression was verified by Western blot analysis and by antiviral activity. The previously described HIV-1 proviral indicator plasmid pNL-Luc-HXB (Luc, luciferase) encodes the *luc* gene in place of the *nef* gene, which is nonessential for HIV-1 replication in culture (21). pNL-Luc-HXB was modified by site-directed mutagenesis to introduce a stop codon at position 26 in the *vif* gene, creating pNL-Luc-HXBΔVif. This mutation has been shown to block

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Abbreviations: agm, African green monkey; agm3G, agm APOBEC3G; HA, influenza hemagglutinin; h3G, human APOBEC3G; HIV-1, HIV type 1; Luc, luciferase; SIV, simian immunodeficiency virus; Vif, virion infectivity factor.

See Commentary on page 3725.

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HIV-1 Vif function (22). The pgVif and pSIVagmVif expression plasmids have been described (19). The pNL4–3ΔEnvΔVif indicator plasmid is similar to pNL-Luc-HXBΔVif, except that it retains the viral *nef* gene and contains a defective *env* gene due to a frame-shift mutation obtained by filling in an *Nde*I site located near the beginning of the *env* ORF.

Virus Production and Luc Assay. 293T cells were cultured in 5% FBS in DMEM and transfected by using the calcium phosphate method, as described (23). Briefly, 1.5 μg of pNL-Luc-HXBΔVif, 250 ng of pgVif or pSIVagmVif, and 125 ng of an APOBEC3G expression plasmid (chimera, mutant, or wild type) were transfected at time 0. Parental plasmids were used as negative controls. At 44 h posttransfection, supernatant media were collected, passed through a 0.45-μm pore-size filter, and then used to infect 293T cells that had previously been transfected with pCMV/CD4 and pCMV/CXCR4 (21). Aliquots of the supernatant were saved and HIV-1 p24 levels measured by using an HIV-1 p24 ELISA Kit (Perkin–Elmer). Approximately 28 h postinfection, cells were washed in PBS and lysed in Passive Lysis Buffer (Promega). Luc activity was then quantitated by using the Promega Luciferase Assay System and light emission measured by using a TD-20/20 Luminometer (Turner, Palo Alto, CA). After harvest of the virus supernatant, the cultures were lysed and the lysate subjected to gel electrophoresis and then transferred to a nitrocellulose membrane. The membranes were then probed with a mouse mAb specific for either the HA epitope tag (Covance, Berkeley, CA) or the HIV-1 p24 Gag protein (24). Reactive proteins were detected by using the Lumi-Light Western blotting substrate (Roche Diagnostics).

Viral Packaging of APOBEC3G Proteins. 293T cells were transfected as described above by using pNL4–3ΔVifΔEnv instead of pNL-Luc-HXBΔVif. At 44 h posttransfection, 9 ml of virus containing supernatant media was layered onto a 2-ml 20% (wt/vol) sucrose cushion in PBS. Virus was pelleted by centrifugation at 35,000 rpm for 2 h at 4°C in an SW41 rotor. Pellets were resuspended and normalized to HIV-1 p24 values determined from the original supernatant. Levels of the virion p24 Gag protein and of the incorporated h3G, h3G(D182K), agm3G, or agm3G(K128D) proteins were then analyzed by Western blotting, as described above.

Immunoprecipitations. 293T cells were transfected with 500 ng of a plasmid expressing a wild-type or mutant APOBEC3G protein and with 1,000 ng of the pgVif vector expressing wild-type HIV-1 Vif. At 48 h posttransfection, the cells were suspended in lysis buffer (50 mM Tris, pH 7.4/150 mM NaCl/0.5% Nonidet P-40). The resultant lysate was clarified by brief centrifugation and half added to a small column loaded with 25 μl of HA affinity matrix (Covance). After binding for 1 h at 4°C, the columns were extensively washed with lysis buffer and equal aliquots of the total and bound fractions subjected to gel electrophoresis and transferred to a nitrocellulose membrane. The membrane was then cut in half and the upper half probed with an HA epitope-tag-specific rabbit polyclonal antibody (Covance), whereas the bottom half was probed with an anti-Vif rabbit polyclonal antiserum (25).

Results

Mariani *et al.* (10) have reported the sequence of APOBEC3G orthologs encoded by agm, rhesus macaque, and chimpanzee. We have also cloned several independent full-length agm3G cDNA clones from the agm kidney cell line CV-1. Alignment of the predicted h3G and agm3G protein sequences reveals that these proteins are 77% identical at the amino acid level (Fig. 6). Nevertheless, it has previously been reported that inhibition of HIV-1 infectivity by h3G is relieved by HIV-1 Vif but not

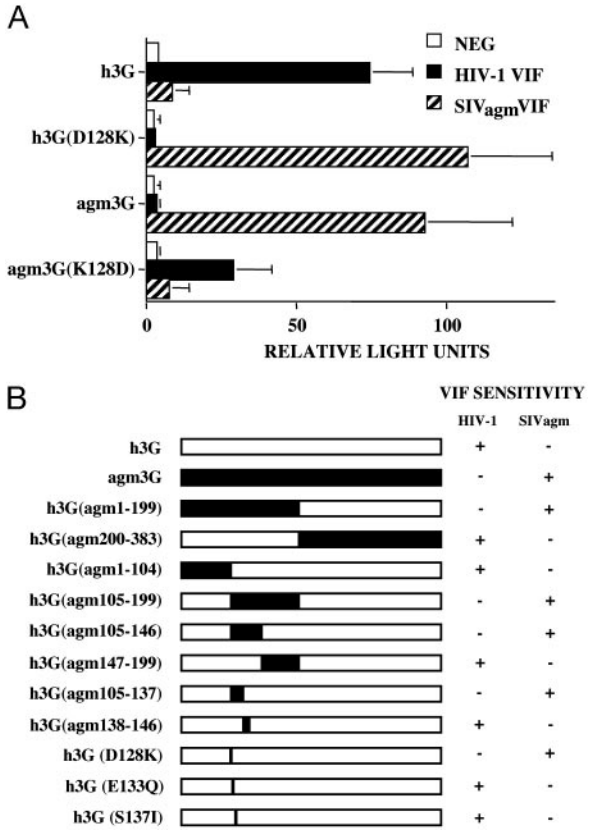

Fig. 1. Mapping the viral tropism determinant in APOBEC3G. (*A*) 293T cells were cotransfected with the pNL-Luc-HXBΔVif proviral indicator construct together with the HIV-1 Vif expression plasmid pgVif or the SIVagm Vif expression pSIVagmVif, or a control plasmid, and finally with plasmids expressing wild-type or mutant forms of h3G or agm3G. At 44 h after transfection, the supernatant media were harvested and used to infect $CD4^+$, $CXCR4^+$ 293T cells. A further 28 h later, the infected cells were lysed and the level of virus-encoded Luc activity was quantified. Data are expressed relative to the level of Luc activity induced by virus derived from a culture transfected with pNL-Luc-HXBΔVif in the absence of any APOBEC3G or Vif expression plasmid, which was arbitrarily set at 100. The average of three independent experiments with SD is indicated. (*B*) Schematic representation of chimeric APOBEC3G proteins derived by substitution of agm3G segments in place of h3G segments. The name indicates which segment of agm3G has been inserted into h3G, e.g., h3G(agm200–383) contains agm3G residues 200–383 substituted for h3G residues 200–384. Each chimeric protein responded fully to one viral Vif protein (indicated by ''+'') but not to the other viral protein (indicated by ''−''). Representative data from this analysis are shown in Fig. 9.

SIVagm Vif, whereas inhibition of HIV-1 infectivity by agm3G can be relieved by SIVagm Vif expression but not by HIV-1 Vif (10). To confirm this result, we used an HIV-1 proviral indicator construct termed pNL-Luc-HXBΔVif. This plasmid encodes a full-length HIV-1 provirus in which the *nef* gene has been replaced with the *luc* indicator gene, whereas the *vif* gene has been inactivated by introduction of a premature translation termination codon. Human 293T cells were cotransfected with pNL-Luc-HXBΔVif and expression plasmids encoding C-terminally HA epitope-tagged forms of either wild-type h3G or agm3G and either HIV-1 Vif or SIVagm Vif. At 44 h after transfection, supernatant media were harvested and used to infect 293T cells engineered to express human CD4 and CXCR4. A further 28 h later, induced Luc activity, indicative of infection by the NL-Luc-HXBΔVif indicator virus, was quantified. This experiment showed (Fig. 1*A*) that inhibition of HIV-1ΔVif infectivity by h3G was indeed relieved only by HIV-1 Vif, whereas inhibition by agm3G was relieved only by the SIVagm

Vif protein. Importantly, the level of virion production by the transfected 293T cells was not affected by expression of either Vif or APOBEC3G protein, as demonstrated by both Western blot analysis of intracellular HIV-1 Gag expression (Fig. 7, which is published as supporting information on the PNAS web site) and direct measurement of the level of supernatant virus production, by using an HIV-1 p24 Gag protein ELISA (Fig. 8, which is published as supporting information on the PNAS web site).

To identify residues in the h3G and agm3G proteins that control responsiveness to these lentiviral Vif proteins, we constructed a series of HA epitope-tagged h3G/agm3G chimeras with the aim of identifying the smallest segment of agm3G that, when substituted into h3G, would inhibit responsiveness to HIV-1 Vif while conferring responsiveness to SIVagm Vif. All chimeras tested were found to behave either like wild-type h3G or like wild-type agm3G; i.e., all were fully responsive to one viral Vif protein and entirely nonresponsive to the other (Fig. 1*B* and Fig. 9, which is published as supporting information on the PNAS web site). Increasingly smaller chimeras initially mapped the tropism determinant to h3G residues 1–199, then to residues 105–199, to residues 105–146, and finally to residues 105–137 (Figs. 1*B* and 9). This segment of agm3G reveals three amino acid differences with h3G, i.e., at positions 128, 133, and 137 (Fig. 6). Substitution of each of these residues in h3G in turn with the equivalent residue in agm3G revealed that replacement of the aspartic acid at position 128 in h3G with lysine (D128K) was sufficient to render h3G entirely refractory to HIV-1 Vif and fully responsive to SIVagm Vif, whereas the mutations at positions 133 and 137 were phenotypically silent (Figs. 1 and 9). Importantly, all h3G/agm3G chimeras were fully able to inhibit the infectivity of HIV-1 in the absence of any Vif protein (Fig. 9), and all chimeras were expressed at equivalent levels in transfected cells in the absence of Vif, as assessed by Western blot analysis by using an antiserum specific for the HA epitope tag (data not shown).

The data presented in Fig. 1 demonstrate that the D128K mutation is sufficient to confer the Vif responsiveness of agm3G on an otherwise wild-type h3G protein, i.e., h3G(D128K) is fully responsive to SIVagm Vif and entirely refractory to inhibition by HIV-1 Vif. We therefore asked whether the converse mutation, i.e., substitution of lysine 128 with aspartic acid in agm3G [agm3G(K128D)], would exert a similarly profound phenotypic effect. In fact, the agm3G(K128D) mutant did acquire a significant level of responsiveness to HIV-1 Vif while becoming entirely refractory to inhibition by SIVagm Vif (Fig. 1*A*). However, the observed phenotype of agm3G(K128D) is not a complete recapitulation of the wild-type h3G phenotype, as was seen with agm3G and h3G(D128K), because inhibition of viral infectivity by agm3G(K128D) was not fully reversed by coexpression of HIV-1 Vif (Fig. 1*A*).

It has recently been reported that the HIV-1 Vif protein directly and specifically binds h3G and then inhibits incorporation of h3G into HIV-1 virions by sequestration and/or by inducing the degradation of h3G by the proteasome (10, 12–18). To test whether the wild-type h3G and agm3G proteins, as well as the minimal h3G(D128K) and agm3G(K128D) chimeras, would be able to bind HIV-1 Vif specifically, we transfected 293T cells with the HIV-1 Vif expression plasmid pgVif together with vectors expressing HA epitope-tagged forms of each APOBEC3G protein. At 48 h after transfection, the transfected cells were lysed and the APOBEC3G proteins immunoprecipitated by using a mAb specific for the HA tag. The immunoprecipitate was then subjected to Western blot analysis by using rabbit polyclonal anti-HA or anti-Vif antisera. As shown in Fig. 2, both wild-type h3G and the agm3G(K128D) mutant were able to coimmunoprecipitate the HIV-1 Vif protein (lanes 3 and 9). In contrast, the h3G(D128K) point mutant and wild-type agm3G failed to interact with HIV-1 Vif (lanes 5 and 7). Therefore, the

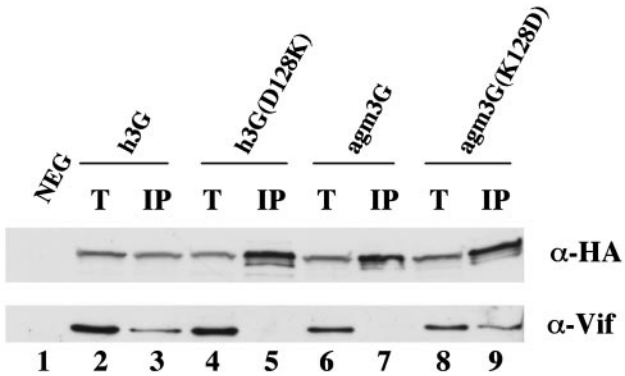

Fig. 2. Binding of HIV-1 Vif by primate APOBEC3G proteins is specific. We transfected 293T cells with the HIV-1 Vif expression plasmid pgVif and a plasmid encoding the indicated HA-tagged APOBEC3G protein. At 48 h after transfection, the cells were lysed and subjected to immunoprecipitation by using a mouse anti-HA mAb. Proteins present in the total lysate (T) or immunoprecipitate (IP) were then separated by gel electrophoresis, transferred to a nitrocellulose filter, and analyzed by Western blot analysis by using rabbit polyclonal antisera specific for the HA tag (*Upper*) or the HIV-1 Vif protein (*Lower*). The negative (Neg) control lane was transfected with the control pcDNA3 plasmid. This experiment used 2% of the total lysate and 10% of the immunoprecipitate in each lane.

ability of these primate APOBEC3G proteins to respond to HIV-1 Vif correlates with their ability to bind HIV-1 Vif specifically. We were unable to perform the converse experiment, using SIVagm Vif, because we do not possess an antiserum specific for this viral protein.

We next asked whether the ability of the viral Vif proteins to counteract the inhibition of viral infectivity caused by APOBEC3G proteins would correlate with a reduction in the level of expression of that protein, as would be predicted if Vif induced its specific degradation. For these experiments, we used the same transfection conditions used in Fig. 1 to visualize the rescue of viral infectivity by Vif proteins. At 48 h, at the same time that the cell supernatants were harvested for assay of viral infectivity, we lysed the producer 293T cells and then performed a Western analysis of APOBEC3G protein expression (Fig. 3), as well as HIV-1 Gag protein expression as an internal control (Fig. 7). These data demonstrated a sharp (>25-fold) drop in the level of expression of the wild-type h3G protein in the presence of HIV-1 Vif and, surprisingly, a 3- to 4-fold reduction in the presence of SIVagm Vif. A similarly steep drop in the level of expression of both the wild-type agm3G and the mutant h3G(D128K) proteins occurred in the presence of the SIVagm but not HIV-1 Vif protein (Fig. 3). Finally, the agm3G(K128D) chimera displayed a moderate, ≈3-fold, decline in expression level in the presence of HIV-1 Vif but was largely unaffected (<2-fold) by SIVagm Vif. In contrast, neither HIV-1 nor SIVagm Vif expression had any effect on the level of HIV-1 Gag expression (Fig. 7). Together, these data suggest that APOBEC3G proteins that are effectively inhibited by coexpression of a viral Vif protein are also at least partially destabilized by that Vif protein.

It may seem contradictory that the level of expression of certain APOBEC3G proteins is strongly reduced by HIV-1 or SIVagm Vif expression in the results reported in Fig. 3, whereas there appears to be little effect of Vif on APOBEC3G expression in the data reported in Fig. 2. This discrepancy arises from the fact that the transfection analyzed in Fig. 2 utilizes 4-fold more of the APOBEC3G expression plasmids and omits the HIV-1 proviral indicator plasmid. Under the latter conditions, which were designed to permit the specific coimmunoprecipitation of h3G and HIV-1 Vif, we observe a significantly higher level of

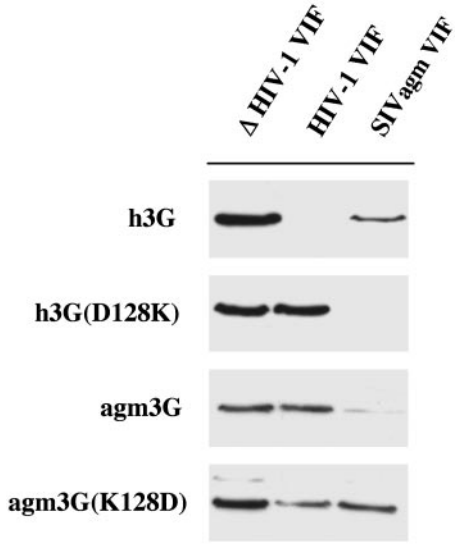

Fig. 3. Specific repression of APOBEC3G expression by HIV-1 or SIVagm Vif. 293T cells were transfected as described in Fig. 1*A*. At the same time that the virus containing supernatant was harvested for analysis, the transfected cell cultures were lysed and the level of expression of the indicated APOBEC3G proteins was determined by Western blot analysis by using a mouse mAb specific for the HA epitope tag. A parallel Western blot analysis measuring Gag protein expression is shown in Fig. 7.

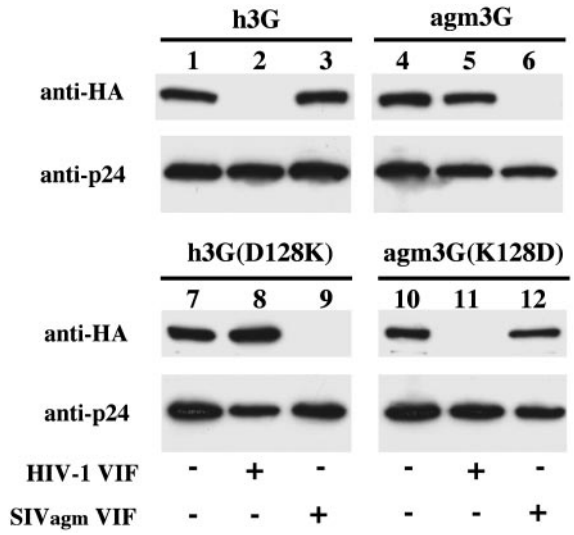

Fig. 4. Primate immunodeficiency virus Vif proteins can selectively block virion incorporation of APOBEC3G. This assay was performed as described in Fig. 1, except that the proviral expression plasmid pNL-Luc-HXBΔVif was replaced with the similar plasmid pNL4–3ΔEnvΔVif. At 44 h, the supernatant media were harvested and the released virions were collected by ultracentrifugation. After lysis, the level of the indicated HA-tagged APOBEC3G proteins incorporated into the virions was determined by Western blot analysis by using a HA-specific mouse mAb. Virion p24 Gag protein levels were measured in parallel.

expression of each APOBEC3G protein that is not strongly affected by Vif coexpression (Fig. 2 and data not shown). Others have also previously noted that small differences in the relative level of Vif and h3G expression plasmids transfected into cells can have a large effect on the observed level of expression of the h3G protein (13).

A key property of the HIV-1 Vif protein is that it blocks the incorporation of h3G into HIV-1 virions and thereby prevents the editing of HIV-1 reverse transcripts during subsequent infections (8–11, 14). We therefore analyzed the effect of HIV-1 and SIVagm Vif expression on the incorporation of the h3G, h3G(D128K), agm3G, and agm3G(K128D) proteins into HIV-1 virions. In these experiments, which were performed essentially as described in Fig. 1, released virions present in the supernatant media were first collected by ultracentrifugation and then subjected to Western analysis by using either a mAb specific for the HA epitope tag present on all of the APOBEC3G proteins or a p24 Gag-specific antiserum. As shown in Fig. 4, HIV-1 Vif, but not SIVagm Vif, effectively blocked both h3G and agm3G(K128D) packaging into HIV-1 virions. Conversely, SIVagm Vif, but not HIV-1 Vif, effectively prevented packaging of both wild-type agm3G and the h3G(D128K) point mutant into HIV-1 virions. Therefore, the ability of each viral Vif protein to rescue the infectivity of HIV-1 virions in the presence of an APOBEC3G protein (Fig. 1) is fully correlated with its ability to block the virion incorporation of that protein (Fig. 4). In contrast, the ability of HIV-1 and SIVagm Vif to block incorporation of APOBEC3G variants into virions correlates only in part with their effect on the intracellular level of expression of these proteins (compare Figs. 3 and 4). The reason for this apparent discrepancy is currently under investigation.

The data presented in this paper argue that the identity of the residue at position 128 in h3G and agm3G can control the ability not only of HIV-1 and SIVagm Vif to inhibit APOBEC3G function (Fig. 1) but also of HIV-1 Vif to bind h3G and agm3G specifically (Fig. 2). This could suggest that formation of APOBEC3G:Vif complexes depends on the formation of an ionic or salt-bridge interaction between these two proteins involving APOBEC3G residue 128. To test this hypothesis, we substituted position 128 in both h3G and agm3G with arginine, which, like lysine, is a basic amino acid; glutamic acid, which is an acidic like aspartic acid; alanine, a small hydrophobic amino acid; and lastly leucine, a large hydrophobic amino acid. As shown in Fig. 5, none of these missense mutations reduced the ability of either h3G or agm3G to block the infectivity of an HIV-1 provirus lacking Vif.

Analysis of the Vif responsiveness of the h3G point mutants to HIV-1 or SIVagm Vif showed that h3G(D128R), like h3G(D128K), was nonresponsive to HIV-1 Vif but, unlike h3G(D128K), acquired only partial susceptibility to SIVagm Vif (Fig. 5). The conservative D128E mutation of h3G gave a phenotype similar to wild-type h3G in that h3G(D128E) was entirely nonresponsive to SIVagm Vif but retained only ≈50% of the ability to respond to HIV-1 Vif. Surprisingly, the h3G(D128A) mutation also retained substantial, albeit not complete, responsiveness to HIV-1 Vif and possibly acquired a weak ability to respond to SIVagm Vif. Finally, the h3G(D128L) mutation was unable to respond to either HIV-1 or SIVagm Vif (Fig. 5).

Analysis of a similar set of agm3G point mutants showed that agm3G(K128R) and agm3G(K128A) were fully responsive to SIVagm Vif, whereas agm3G(K128E), unlike agm3G(K128D), appeared to be slightly responsive to SIVagm Vif (Fig. 5). In addition to the agm3G(K128D) mutant, partial responsiveness to HIV-1 Vif was also detected for agm3G(K128E) and, particularly, agm3G(K128A). As in the case of h3G, substitution of position 128 in agm3G with leucine blocked responsiveness to either lentiviral Vif protein (Fig. 5).

Discussion

The data presented in this paper demonstrate that a single residue difference in APOBEC3G, which is an aspartic acid in humans and chimpanzees and a lysine in agm and rhesus macaques (Fig. 6), accounts for the inability of both HIV-1 Vif to function in agm cells and SIVagm Vif to function in primary human cells. Although there is at least one additional factor that

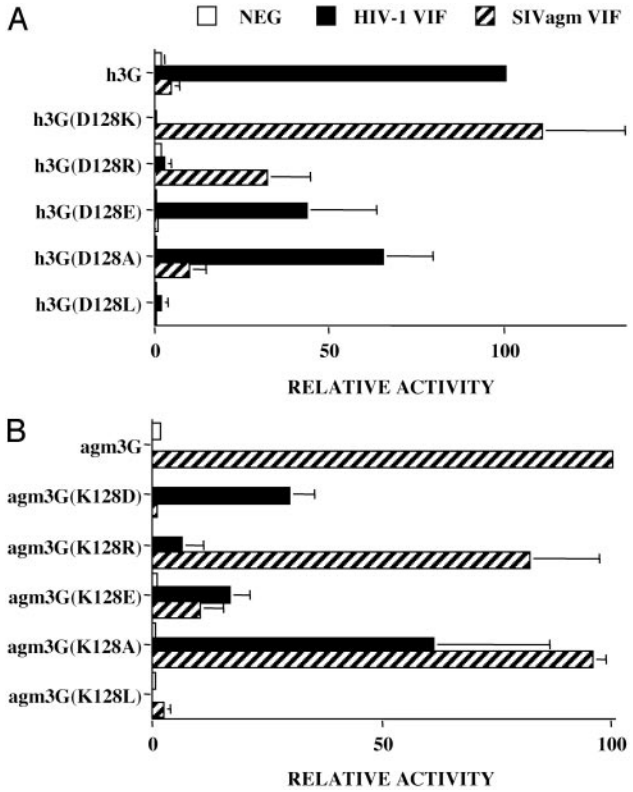

Fig. 5. Mutagenesis of residue 128 in h3G and agm3G. This determination of the ability of point mutants of h3G (*A*) or agm3G (*B*) to inhibit the infectivity of Vif-defective HIV-1 and to respond to the HIV-1 Vif or SIVagm Vif protein was performed as described in Fig. 1. Data are expressed relative to the level of Luc activity induced by virus derived from a culture cotransfected with pNL-Luc-HXBΔVif and either h3G and HIV-1 Vif (*A*) or agm3G and SIVagm Vif (*B*), which was set at 100. The average of three independent experiments with SD is indicated.

can restrict infection of simian cells by HIV-1 (26, 27), no such additional factor is known to restrict infection of human cells by SIVagm. Indeed, SIVagm is similar to Vif-negative HIV-1 mutants in that it is able to grow effectively in transformed human cell lines that express human CD4 and CXCR4 but not h3G, whereas primary human peripheral blood mononuclear cells, which do express h3G, are nonpermissive for both SIVagm and Vif$^-$ HIV-1 (7, 20, 28). These data therefore suggest that a single residue in h3G may be the critical factor that has blocked SIVagm, which is widespread in a monkey species common in Africa, from following the chimpanzee variant of SIV and sooty mangabey SIV and becoming a zoonotic infection of humans. Unfortunately, the demonstration that SIVagm Vif function in human cells is blocked by the identity of a single residue in h3G implies that relatively minor sequence changes in SIVagm Vif might be able to overcome this biological barrier and thereby allow SIVagm to adapt to human hosts. Although this possibility is clearly of concern, our data also suggest that it may be possible to construct or select a mutant of HIV-1 Vif that can down-regulate simian APOBEC3G proteins, including those encoded by agm and rhesus macaques, that bear a lysine residue at position 128 (10) (Fig. 6). Such HIV-1 mutants could potentially be very useful reagents in the study of HIV-1 pathogenesis in experimental animals.

It has recently been reported that a fragment of h3G, consisting of residues 54–124, is able to specifically interact with HIV-1 Vif in transfected cells (12), thus implying that h3G residue 128 cannot be a key mediator of this interaction. Consistent with this suggestion, mutation of D128 to alanine in h3G, or of K128 to alanine in agm3G, did not block the ability of these proteins to respond to their cognate Vif protein and indeed conferred substantial responsiveness to HIV-1 Vif on the agm3G protein (Fig. 5). We therefore hypothesize that position 128 may not directly participate in Vif:APOBEC3G binding, but rather that the presence of an inappropriate residue at this location, i.e., a residue bearing an inappropriate charge or a large hydrophobic residue such as leucine (Fig. 5), interferes with complex formation. Of course, a final determination of the role of residue 128 in mediating the formation of the Vif:h3G complex will likely require the derivation of an x-ray crystallographic structure of this complex.

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