APOBEC3B and APOBEC3C Are Potent Inhibitors of Simian Immunodeficiency Virus Replication*

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In the human genome the apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC) gene has expanded into a tandem array of genes termed APOBEC3A-G. Two members of this family, APOBEC3G and APOBEC3F, have been found to have potent activity against simian immunodeficiency virus 1 (SIV-1). These enzymes become encapsidated in SIV-1 virions and in the next round of infection deaminate the newly synthesized reverse transcripts. The lentiviral Vif protein prevents the deamination by inducing the degradation of APOBEC3G and APOBEC3F. We report here that two additional APOBEC3 family members, APOBEC3B and APOBEC3C, have potent antiviral activity against simian immunodeficiency virus (SIV), but not HIV-1. Both enzymes were encapsidated in HIV-1 and SIV virions and were active against Vif SIVmac and SIVagm. SIV Vif neutralized the antiviral activity of APOBEC3C, but not that of APOBEC3B. APOBEC3B induced abundant G → A mutations in both wild-type and Vif SIV reverse transcripts. APOBEC3C induced substantially fewer mutations. APOBEC3F was found to be active against SIV and sensitive to SIVmac Vif. These findings raise the possibility that the different APOBEC3 family members function to neutralize specific lentiviruses.

Apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC)-like 3G (APOBEC3G) and 3F (APOBEC3F) are cellular cytidine deaminases that belong to the APOBEC family of RNA-editing and DNA mutator proteins (reviewed in Ref. 1). In cells infected with HIV-1 that is deleted for the virion infectivity factor (vif) gene (Δvif), APOBEC3G and APOBEC3F become encapsidated during virus assembly, and the virions that are released are greatly reduced in infectivity (2–9). These encapsidated APOBEC proteins deaminate the viral reverse transcripts upon minus-strand synthesis; as a result, virus replication is aborted prior to integration (3–7). Much of the deaminated DNA appears to be degraded by cellular DNA repair enzymes prior to integration. Those molecules that escape degradation are compromised in their coding capacity as a result of G → A mutations that insert frequent termination codons throughout the viral genome (10). In cells infected with wild-type HIV-1, Vif rescues viral infectivity by binding to APOBEC3G and APOBEC3F, inducing their polyubiquitination and proteasomal degradation through an association with an E3 ubiquitin ligase consisting of Elongin B/C, Rbx-1, and Cul5 (11–14).

The interaction of Vif with APOBEC3G is species-specific (4, 15, 16). HIV-1 Vif binds to human APOBEC3G but not mouse, African green monkey (AGM), or rhesus macaque (MAC) APOBEC3G (4). As a result, noncognate APOBEC3G proteins tend to block the replication of both Δvif and wild-type HIV-1. Conversely, Vif of SIVmac but not SIVagm binds to human APOBEC3G; therefore, human APOBEC3G inhibits wild-type SIVagm but not wild-type SIVmac Vif (4). The species specificity of the Vif-APOBEC3G interaction is determined by a single amino acid difference in APOBEC3G at position 128 that encodes a charged amino acid (16–19). The encapsidation of APOBEC3G into retroviral virions is less virus-specific. APOBEC3G can be encapsidated by HIV, SIV, and murine leukemia virus (MLV) through a mechanism thought to involve binding to viral RNA, cellular RNA, or the viral nucleocapsid protein (20–23). APOBEC3G can also be encapsidated in hepatitis B virus and is thought to inhibit replication without deamination of the viral DNA (24).

In the human genome, the APOBEC gene family consists of APOBEC1, APOBEC2, activation-induced deaminase (AID), and APOBEC3A-G (here referred to as APOBEC3 in general) (25). The mouse genome contains only a single APOBEC3 gene, suggesting that APOBEC3A-G is a fairly recent evolutionary expansion (26). Each APOBEC protein contains a catalytic domain characterized by a conserved acidic motif that shields a His and two Cys residues that coordinate a Zn2+ and a Glu residue that acts as a proton shuttle during catalysis. In APOBEC3B, APOBEC3F, and APOBEC3G the structural unit has been duplicated such that the enzyme contains two potential catalytic domains (25). In addition, the APOBEC proteins are generally dimeric.

APOBEC3G was the first cytidine deaminase shown to have anti-lentiviral activity (2). Recently, its most closely related family member, APOBEC3F, was also shown to be active against HIV-1 and a target of Vif (8, 9, 27, 28). APOBEC3F is expressed in lymphocytes where it is able to form heteromul-
timers with APOBEC3G (9, 27). The two enzymes differ in target sequence preference, in that APOBEC3F prefers TC, whereas APOBEC3G targets C (9, 27, 28). Together such sites appear to be the major contributors to HIV-1 hypermutation in vivo.

Here, we characterized APOBEC3B and APOBEC3C for their activity against HIV-1, SIVmac and SIVagm. Although they were hardly active against HIV-1, APOBEC3B and APOBEC3C were potent inhibitors of SIV. Both enzymes were efficiently encapsidated by HIV-1 and SIV. These findings raise the possibility that the various APOBEC3 family members protect against different lentiviruses and provide a rationale for the expansion of the gene family in primates.

**Experimental Procedures**

**APOBEC Expression Vectors—**APOBEC3B expression vector was derived by cloning the full-length cDNAs into the pcDNA3.1(+) (Invitrogen). The cDNAs were generated by reverse transcriptase PCR using the RNA isolated from phytohemagglutinin-activated human peripheral blood mononuclear cells with RNasey kit (Qiagen). The cDNA was primed with oligo(dT) and extended with Superscript III reverse transcriptase (Invitrogen). The APOBEC3B expression vector was constructed with primers that contained EcoRI and SalI restriction sites and encoded the S′-influenza hemagglutinin (HA) tag. T7 poly(P) promoter and 3′-poly(A)tail in which the target sequence preference was replaced with U was processed in parallel to indicate the position of the cleaved product.

**Real-time PCR Quantitation of APOBEC3 mRNA in Primary Cell Populations—**Peripheral blood mononuclear cells were separated from healthy donor blood by Ficoll (Amersham Pharmacia Biotech) centrifugation. To purify unactivated CD4+ T cells, peripheral blood mononuclear cells were positively sorted on anti-CD4 Dynabeads and removed with 10 μg/ml soluble anti-CD4 antibody (Dynal Biotech) as previously described (32). The cells were further purified by negative selection on anti-CD8 and anti-CD14 (BD Biosiences) with goat anti-mouse IgG (Dynal Biotech)-coated beads. The purified cells were 99.5% CD4+ as determined by flow cytometry. Activated cells were prepared as previously described (33).

Monocytes were purified from peripheral blood mononuclear cells by positive selection on anti-CD14-coated magnetic beads (Miltenyi Biotech) using an AutoMacs (Miltenyi Biotech). The monocytes were confirmed by sequencing. A 1.1-kb fragment covering a portion of the env and nef (nucleotides 8496–9069 in NL4–3 and 8153–9525 in SIVagm) was amplified with high fidelity DNA polymerase (Roche Applied Science) and cloned into the TOPO TA-cloning vector pCR4 (Invitrogen). The nucleotide sequence of at least 10 independent clones was analyzed using Hypermut software (31). The primers used for PCR and sequencing were SIVenv-F, 5′-GCC GTC GAT TTT CAA CAA TAC ACC; SIVagg-R; 5′-GCT TCC TGG GAC ACA TCA GCT ACC; HIV-1-F, 5′-GGA GCC TGT GCC TCA TCA GCT ACT GCC; HIV-1-R, 5′-GCT TTA TGG AGG CTT AAG CAG TGG T.

**Cytidine Deaminase Assay—**This assay was modified from the deaminase activity assay for encapsidated APOBEC3G previously described (10). Briefly, virions were pelleted and solubilized in 100 μl of virus lysis buffer containing 0.1% (w/v) Triton X-100 (10). Virus lysate containing 100 ng of p24 was mixed with 1 10× cmpt of 5′-end-labeled oligonucleotide containing the indicated target sequence in the deaminase buffer. After 5 h at 37 °C, the reactions were heated to 90 °C for 5 min and then incubated with uracil DNA glycosylase (UDG) (New England Biolab) in UDG buffer for another 30 min at 37 °C. Subsequently, the reaction was brought to 0.15 μM NaOH at 37 °C for 30 min. The products were separated on a precast 15% TBE-urea PAGE (Invitrogen) and detected by autoradiography. A labeled marker oligonucleotide was used to ensure that the oligonucleotide had a complete deamination.

**Results**

**HIV-1 and SIV Encapsidate APOBEC3B and APOBEC3C—**To determine whether different APOBECs can be encapsidated by lentiviruses, virions were generated by cotransfection of 293T cells with wild-type or Δ3if HIV-1 or SIVagm proviral clones and expression vectors for HA-tagged APOBECs. Virus-containing culture supernatant was harvested 2 days posttransfection, the virions were pelleted by ultracentrifugation, normalized for p24/p27 by enzyme-linked immunosorbent assay, and then analyzed for APOBEC content by immunoblot analysis with anti-HA mAb. To determine the relative expression levels of the APOBEC proteins, lysates of the transfected

**Sequence of Viral Reverse Transcripts—**HOS.CD4.x4 cells (1 × 106) were infected with DNase-I-treated HIV-1 or SIVagm (50 ng of p24/p27). After 4 h, the cells were washed with medium; at 12 h postinfection, DNA was isolated using DNeasy DNA isolation kit (Qiagen). A 1.1-kb fragment covering a portion of the env and nef nucleotides was amplified with high fidelity DNA polymerase (Roche Applied Science) and cloned into the TOPO TA-cloning vector pCR4 (Invitrogen). The nucleotide sequence of at least 10 independent clones was analyzed using Hypermut software (31). The primers used for PCR and sequencing were SIVenv-F, 5′-GCC GTC GAT TTT CAA CAA TAC ACC; SIVagg-R; 5′-GCT TCC TGG GAC ACA TCA GCT ACC; HIV-1-F, 5′-GGA GCC TGT GCC TCA TCA GCT ACT GCC; HIV-1-R, 5′-GCT TTA TGG AGG CTT AAG CAG TGG T.
cells were prepared and analyzed similarly on immunoblots (Fig. 1A). The results showed that APOBEC3B and APOBEC3C were encapsidated into HIV-1 and SIVagm virions (Fig. 1B). Both enzymes were more efficiently encapsidated than APOBEC3G in HIV-1 and SIV. Controls with human and AGM APOBEC3G demonstrated the expected species specificity; HIV-1, but not SIVagm, Vif, reduced the amount of human APOBEC3G that was encapsidated. Conversely, SIVagm, not HIV-1 Vif, reduced the AGM APOBEC3G encapsidation. This species specificity did not apply to APOBEC3B and APOBEC3C. HIV-1 and SIVagm Vif reduced the amount of encapsidated APOBEC3C, but not APOBEC3B (Fig. 1B). Two other members of the APOBEC family, APOBEC2 and AID, were also efficiently incorporated into wild-type and Δvif HIV-1 virions (Fig. 1B). There was slightly more APOBEC3B and AID in the wild-type as compared with Δvif virus particles, a phenomenon previously noted for mouse and AGM APOBEC3G encapsidated into HIV-1 virions (4). HIV-1 Vif appeared to slightly reduce the amount of encapsidated APOBEC2.

**APOBEC3B and APOBEC3C Inhibit SIV Infectivity**—Although the different APOBECs could be encapsidated in HIV-1 and SIV, it was not clear whether they would have antiviral activity. To determine this, VSV-G pseudotyped wild-type and Δvif HIV-1, SIVagm, and SIVmac luciferase reporter viruses were prepared in 293T cells cotransfected with APOBEC expression vectors. The infectivity of the viruses was determined by infecting HOS cells with viruses normalized for p24/p27 and measuring luciferase activity 3 days postinfection (Fig. 2). The amount of APOBEC3G expression vector in the transfection was detected on an immunoblot probed with anti-HA mAb. B, encapsidated APOBEC in virions normalized by p24/p27 enzyme-linked immunosorbent assay was detected on a parallel immunoblot, labeled as in panel A. The presence or absence of Vif in the viruses is indicated as + and −, respectively. Equal loading of virions on the immunoblots was confirmed with antibody to the HIV and SIV capsid.

**Fig. 1.** APOBEC3B and APOBEC3C are encapsidated into HIV-1 and SIVagm virions. Virions were generated by cotransfection of 293T cells with wild-type or Δvif HIV-1 or SIVagm DNA and HA-tagged APOBEC expression vector. Human APOBEC3B, APOBEC3C, APOBEC3G, AGM APOBEC3G, and APOBEC2 are labeled h3B, h3C, h3G, agm3G, and APO2, respectively, in this and subsequent figures. A, APOBEC expression in the transfected cells was detected on an immunoblot probed with anti-HA mAb. B, encapsidated APOBEC in virions normalized by p24/p27 enzyme-linked immunosorbent assay was detected on a parallel immunoblot, labeled as in panel A. The presence or absence of Vif in the viruses is indicated as + and −, respectively. Equal loading of virions on the immunoblots was confirmed with antibody to the HIV and SIV capsid.
similar to that on SIV<sub>agm</sub> (Fig. 2, B and C). Human APOBEC2 and AID did not affect the infectivity of HIV-1, SIV<sub>agm</sub>, and SIV<sub>mac</sub> (Fig. 2, A–C).

**Encapsidated APOBEC3B and APOBEC3C Deaminate the Reverse Transcripts**—APOBEC3G and APOBEC3F mediate their antiviral activity by single-strand DNA deamination (9, 10, 27, 28, 34). To determine whether APOBEC3B and APOBEC3C also act through this mechanism, the sequence of viral reverse transcripts from newly infected cells was analyzed. VSV-G pseudotyped wild-type and Δvif HIV-1 and SIV<sub>agm</sub> with or without encapsidated APOBEC were prepared, normalized for p24, treated with DNase I, and then used to infect HOS cells. At 12 h postinfection, a fragment of the reverse transcript that contained the 3′-portion of env and 5′-portion of nef, a region that is highly susceptible to APOBEC3G deamination (10), was amplified and cloned. Nucleotide sequences were determined for at least 10 independent clones from each infection.

APOBEC3B and APOBEC3C generated a low frequency of G → A mutations on HIV-1 reverse transcripts (Fig. 3A). The number of mutations induced by APOBEC3C, but not APOBEC3B, was reduced by Vif. In contrast, APOBEC3B generated a high frequency of G → A mutations on SIV<sub>agm</sub> reverse transcripts (Fig. 3A, quantitated in Fig. 3B). This mutational

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**FIG. 3.** APOBEC3B and APOBEC3C induce G → A mutations in HIV-1 and SIV<sub>agm</sub> reverse transcripts. A, a fragment in env-nef was amplified from HIV-1 and SIV<sub>agm</sub> reverse transcripts at 12 h postinfection. At least 10 independent HIV-1 and SIV<sub>agm</sub> nucleotide sequences were determined. The mutations in eight clones of each group are shown. Each mutation is denoted by a vertical line, color coded with respect to dinucleotide context: GG → AG (red), GA → AA (cyan), GC → AC (green), GT → AT (magenta), and non-G → A (black). B, the percentage of G nucleotides that were changed to A is plotted as the average of the sequenced clones from each SIV<sub>agm</sub> infection. C, the relative frequency of GG → AG, GA → AA, GC → AC, and GT → AT in SIV<sub>agm</sub> generated by APOBEC3B, APOBEC3C, and APOBEC3G is shown graphically as the percentage of G → A mutations in the indicated dinucleotide context.
FIG. 4 Catalytic activity of encapsidated APOBEC3B and APOBEC3C. A, cytidine deaminase activity released from pelleted HIV-1 virions containing the indicated APOBEC3 was measured with 32P-labeled deoxyoligonucleotide containing the target sequence CCCA. The deaminated product is indicated by an arrow. Virions prepared with no APOBEC were used as a control for background activity (lanes 1 and 2). B, cytidine deaminase activity in SIVagm virions. An oligonucleotide containing a dU in place of the target site preference of the SIVagm-encapsidated APOBEC was tested on labeled deoxyoligonucleotides with the sequence CCA, CCT, or CCG. C, the target site preference of the SIVagm-encapsidated APOBEC3B and APOBEC3C was tested on single-stranded DNA (ss), double-stranded DNA (ds), or RNA/DNA hybrid (R/D). Double-stranded substrates were formed by annealing the labeled CCCA deoxyoligonucleotide to unlabeled complementary DNA or RNA.

A PO BE C3 B and A POBE C3 C Inhibit SIV

frequency was comparable with that induced by human APOBEC3G and was not affected by Vif. The pattern of bases changed by APOBEC3B and APOBEC3G was overlapping, with many shared hot spots, but APOBEC3B displayed a broader target sequence preference, targeting both GG and GA dinucleotides (Fig. 3C). APOBEC3C also induced G → A mutations on Δvif SIVagm reverse transcripts, but with a 9-fold lower frequency compared with APOBEC3B (Fig. 3B). These mutations were randomly distributed and lacked obvious hot spots or strong dinucleotide sequence preference (Fig. 3, A and C). Vif reduced the number of mutations by APOBEC3C to near background (Fig. 3, A and B). Taken together, these findings suggest that APOBEC3B and APOBEC3C, as APOBEC3G, act by deaminating minus-strand reverse transcripts.

Deaminase Activity of APOBEC3B and APOBEC3C in Vitro—To investigate the basis for the selective inhibition of SIV, but not HIV-1 infectivity by APOBEC3B and APOBEC3C, the deaminase activity of the encapsidated enzymes was measured in an in vitro assay (10). APOBEC3-containing virions were produced in transfected 293T cells, pelleted, and solubilized. The virion lysate was then incubated with a 5′-end-labeled oligonucleotide containing the sequence CCCA, the favored target site for APOBEC3G (10, 35). The deaminated oligonucleotide was subsequently cleaved at the deamination site by uracil DNA glycosylase and high pH treatment, and the cleaved product was visualized by autoradiography. Despite the much reduced antiviral activity of APOBEC3B against HIV-1 as compared with SIVagm, the cytidine deaminase activity of APOBEC3B released from HIV-1 and SIVagm virions was similar (Fig. 4, A and B). Thus, the APOBEC3B encapsidated by HIV-1 remained catalytically active but the HIV-1 genome was largely resistant to it.

APOBEC3B was highly active on the target sequence CCCA (Fig. 4, A and B). In contrast, APOBEC3C was weakly active against CCAA. The target sequence preference of the APOBECs was further analyzed by testing labeled oligonucleotide substrates that contained CCA, CCT, and CCG target sites (Fig. 4C). These are suboptimal targets for human and AGM APOBEC3G. As expected, human and AGM APOBEC3G were weakly active on these sequences in vitro (Fig. 4C). In contrast, APOBEC3B was highly active against each of these targets. APOBEC3C was poorly active on each substrate. These findings are consistent with the pattern of the mutations generated by APOBEC3B and APOBEC3C on the SIV reverse transcripts. The strand specificity of APOBEC3 was determined using single-stranded (ss) DNA, double-stranded (ds) DNA, and RNA-DNA hybrid as substrates in the assay. APOBEC3B was specific for ssDNA but not dsDNA or RNA-DNA hybrids, similar to APOBEC3G (Fig. 4D). APOBEC3C was also inactive against dsDNA and RNA-DNA hybrid.

MLV Was Relatively Resistant to APOBEC3B and APOBEC3C—We previously reported that APOBEC3G was efficiently encapsidated in MLV virions but had only a small effect on the infectivity of an MLV-EGFP reporter virus (4). To determine whether MLV might be sensitive to APOBEC3B or APOBEC3C, MLV-EGFP reporter viruses were prepared in 293T cells cotransfected with APOBEC3 expression vector. The virions were then harvested and tested for APOBEC3 encapsidation, infectivity, and deaminase activity. Human APOBEC3B, APOBEC3C, APOBEC3G, and AGM APOBEC3G were found to be encapsidated in MLV virions (Fig. 5A). Nonetheless, the APOBECs had only a small effect on infectivity of the virus (Fig. 5B). The encapsidated APOBEC3B was enzymatically active as shown in the in vitro deaminase assay (Fig. 5C). In particular, APOBEC3B-containing virions displayed more deaminase activity than APOBEC3G-containing MLV or HIV-1 particles. The activity of the MLV-encapsidated APOBEC3C and human/AGM APOBEC3G was relatively low in this assay.

APOBEC3F Is Active against SIV—In light of the virus specificity of the APOBEC3 family members, we tested APOBEC3F against SIV. As previously reported, APOBEC3F was active against HIV-1 but with a reduced sensitivity to Vif compared with APOBEC3G (Fig. 6A) (8, 9, 27, 28). APOBEC3F was active against SIVagm, as was APOBEC3G, but both were resistant to SIVagm Vif (Fig. 6B). APOBEC3F was also active against Δvif SIVmax, but as with APOBEC3G it was sensitive to SIVmax Vif (Fig. 6C).

APOBEC3B and APOBEC3C Are Expressed in Lymphoid and Myeloid Cells—APOBEC3B and APOBEC3C would only be relevant to lentiviral replication if they were expressed in lymphoid or myeloid cells, the natural targets of the viruses. To measure expression of these genes in lymphocytes and monocytes, resting and activated human CD4+ T cells and macrophages were prepared, and the expression of APOBEC3B, APOBEC3C, APOBEC3F, and APOBEC3G was analyzed by...
quantitative RT-PCR. The expression of these genes in human heart, small intestine, and the human embryonic carcinoma cell line NCCIT was also analyzed. APOBEC3B and APOBEC3C were expressed in heart and small intestine, whereas only APOBEC3B was expressed in NCCIT cells. APOBEC3F and APOBEC3G were expressed at low levels in the nonlymphoid cells tested. Each of these genes was expressed in activated CD4⁺ T cells, and their expression was increased upon T cell activation (Fig. 7). These genes were also expressed in macrophages, whereas the level of APOBEC3F expression was relatively low. These results were consistent among all three donors. Thus, APOBEC3B and APOBEC3C are expressed in lymphoid cells where they could play a role in restricting retroviral replication.

**DISCUSSION**

We report here that two of the human APOBEC3 family members, APOBEC3B and APOBEC3C, have potent antiviral activity against two types of SIV, SIVmar and SIVagm. Interestingly, these deaminases were only marginally active against HIV-1 despite that they were efficiently encapsidated. APOBEC3F, which has been previously reported to inhibit HIV
(8, 9, 27, 28), was also active against SIV. The specificity of the antiviral activity was not caused by differences in encapsidation, as APOBEC3B and APOBEC3C were encapsidated at relatively high levels in HIV-1, SIV, and MLV particles. Other APOBEC family members, such as AID and APOBEC2, were also encapsidated in HIV-1 and SIV but did not inhibit these viruses despite their single-strand DNA deaminase activity (8).

APOBEC3B was the most potent deaminase of the APOBEC family members that were tested. Like APOBEC3F and APOBEC3G, APOBEC3B has the duplicated, two-catalytic domain structure. In APOBEC3G it is the C-terminal catalytic domain that mediates deamination (36). The C-terminal catalytic domain of APOBEC3B is unique among APOBEC enzymes in having the two Zn$^{2+}$-coordinating cysteines separated by four amino acids instead of two. This spacing could result in a catalytic site that is less sequence-specific and more active. It is also interesting to note APOBEC3C is a single-unit-length enzyme and is efficiently encapsidated. Thus, it is possible for a single unit to contain sites for both encapsidation and enzymatic activity.

Whether APOBEC3B or APOBEC3C is active in vivo against HIV or SIV remains to be determined. However, it is clear that these enzymes were expressed in CD4$^+$ T cells and macrophages, although their expression level appeared to be lower than that of APOBEC3G. Even though neither of the enzymes was strongly active against HIV-1, the resistance of APOBEC3B to Vif and its potent deaminase activity could allow it to contribute to the G $\rightarrow$ A hypermutation rate of the virus.

The virus specificity of the APOBEC3 antiviral activity is both surprising and difficult to explain. This effect was particularly pronounced for APOBEC3B, which is a potent deaminase that was efficiently encapsidated in HIV-1 yet caused only a few G $\rightarrow$ A changes in the reverse transcripts. In contrast, it generated a high frequency of mutations in SIV. During the preparation of this report, Bishop et al. (28) reported that APOBEC3B was active against HIV-1, but not MLV, unlike APOBEC3G that inhibited both viruses. We reported that both APOBEC3B and APOBEC3G were relatively ineffective against MLV regardless of their efficient encapsidation, reflecting again a specificity for particular retroviruses. Overall, SIV appeared to be most sensitive to APOBEC deamination among the viruses tested. SIV$_{mac}$ and SIV$_{agm}$ were susceptible to APOBEC3B, APOBEC3C, APOBEC3F, and APOBEC3G. HIV-1 was intermediate, being sensitive to APOBEC3F and APOBEC3G, whereas MLV was largely resistant to all of the deaminases tested. The APOBEC enzymes encapsidated in these virions were shown to be enzymatically active, arguing against a viral mechanism for silencing the enzyme. Furthermore, our findings suggest that the simple presence of an active deaminase in the virion is not sufficient to mediate viral reverse transcript deamination. AID, for example, is a highly
active single-strand-specific cytidine deaminase and is efficiently encapsidated in HIV-1 but does not have detectable antiviral activity.

The molecular basis for the differences in virus susceptibility to the different APOBEC3 family members function to restrict the replication of specific lentiviruses, or of other viruses that go through a single-strand DNA intermediate. If this were the case, it would explain the fairly recent evolutionary expansion of the APOBEC3 family in primates, as the expanded family would more effectively protect against diverse retroviruses. In rodents, which are thought to be subject to lentiviral infection, the selective pressure to expand APOBEC3 would have been absent. One possible scheme for the evolution of the gene family is that APOBEC3 originally served a physiological role in the cell, separate from its antiviral function. With the appearance of diverse lentiviruses, the gene may have expanded in primates to more effectively neutralize diverse lentiviruses. Diversification of the APOBEC3 family would make it more difficult for a single Vif to adapt to bind the different proteins. However, this antiviral mechanism was not entirely successful, judged by the wide distribution of primate and human lentiviruses.

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