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Human APOBEC3 Proteins, Retrovirus Restriction, and HIV Drug Resistance

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Abstract

Over 40 million people worldwide currently have HIV/AIDS. Many antiretroviral drugs have proven effective, but drug-resistant HIV variants frequently emerge to thwart treatment efforts. Reverse transcription errors undoubtedly contribute to drug resistance, but additional significant sources of viral genetic variation are debatable. The human APOBEC3F and APOBEC3G proteins can potently inhibit retrovirus infection by a mechanism that involves retroviral cDNA cytosine deamination. Here we review the current knowledge on the mechanism of APOBEC3-dependent retrovirus restriction and discuss whether this innate host-defense system actively contributes to HIV genetic variation.

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Key words

APOBEC3F. APOBEC3G. HIV. Drug resistance. Innate immunity. Retrovirus restriction. Vif.

The HIV ΔVif phenotype

Nearly all lentiviruses encode an accessory protein called the virion infectivity factor (Vif). This Vif is required for HIV-1 replication in primary CD4+ T-cells, monocyte-derived macrophages, and a limited number of CD4+ T-cell leukemia lines, including CEM and H9[1-4]. Cells that will not support the replication of Vif-deficient HIV are termed "nonpermissive". Vif is also required for the replication of simian immunodeficiency virus (SIV) in rhesus macaques[5]. In contrast, Vif function is not required for HIV replication in several other CD4+ T-cell lines[3,4,6,7]. Examples include CEM-SS and SupT1, and these cell lines are correspondingly termed "permissive". Interestingly, HIV ΔVif virions produced from permissive cells are able to infect nonpermissive cells, but those produced from nonpermissive cells are incapable of productive infection[3,4,6,7]. These descriptions imply that Vif is essential in the virus-producing cells, but its importance only becomes apparent when the newly produced virions attempt to infect new target cells.

The aforementioned observations suggested two alternative hypotheses to explain the function of Vif. Either the permissive cells contain a factor that could provide a Vif-like function required for HIV ΔVif replication (complementation), or nonpermissive cells contain a cellular factor that prevents HIV ΔVif replication (inhibition).

These hypotheses were distinguished in cell-fusion experiments, in which permissive and nonpermissive cells were fused and the resulting heterokaryons were used for HIV ΔVif infectivity studies[8,9]. Two outcomes were envisaged. First, if the heterokaryons elicited a permissive phenotype, this would indicate that permissive cells contained a factor that compensated for the absence of Vif. Second, if the heterokaryons displayed a nonpermissive phenotype, this would suggest that nonpermissive cells expressed an activity capable of dominantly inhibiting the replication of Vif-deficient viruses.

The Malim and the Kabat groups showed independently that the resulting heterokaryons were nonpermissive for growth of HIV ΔVif, thereby strongly indicating

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the presence of a dominant antiviral factor[8,9]. These experiments further suggested that Vif would function to neutralize the negative effect of this host-cell factor.

APOBEC3G – a dominant factor that renders cells nonpermissive for HIV ΔVif infection

In 2002 Malim, et al. used a cDNA subtraction strategy to identify transcripts that were expressed preferentially in nonpermissive cells[10]. One of the cDNA was found to encode APOBEC3G. Elegant experiments demonstrated that APOBEC3G expression was sufficient to render permissive cells unable to support the replication of HIV ΔVif. Moreover, northern analyses indicated that APOBEC3G mRNA was expressed strongly in nonpermissive cells, but weakly or not at all in permissive cells. Together with experiments indicating that APOBEC3G could be incorporated into viral particles, Malim, et al. provided a dataset demonstrating that APOBEC3G was indeed a dominant factor capable of inhibiting the replication of Vif-deficient HIV.

Prior studies had revealed that APOBEC3G was encoded on human chromosome 22, and that it belonged to a larger family of potential nucleic acid cytosine deaminases[11]. APOBEC3G had strong amino acid sequence similarity to the well-characterized *apoB* mRNA editing protein, APOBEC1[12]. This likeness suggested that the potent antiretroviral phenotype of APOBEC3G might be mediated by a similar RNA cytosine deamination mechanism[10]. However, APOBEC3G also showed strong amino acid similarities to activation-induced cytidine deaminase, a DNA cytosine deaminase that uses this activity to trigger several antibody gene diversification processes[13,14] as recently reviewed[15-17]. Moreover, several members of this nucleic acid cytosine deaminase family, including APOBEC1 and APOBEC3G, were shown to possess potent DNA cytosine deamination activity in an *E. coli*-based mutation assay[18].

Taken together with the fact that some patient-derived HIV proviral DNA sequences had been found to contain plus-strand-specific G → A transition mutations[19-22], which could be considered equivalent to minus-strand C → T transitions, several groups were attracted to a model in which APOBEC3G inhibited HIV ΔVif replication by deaminating cytosines to uracils in nascent retroviral cDNA[23-26].

A model for HIV restriction by APOBEC3G is depicted in figure 1 (top panel). This model has two key predictions. First, it predicts that APOBEC3G expressed in the nonpermissive virus-producing cell will have the capacity to access new virus particles. This prediction was partly satisfied by the original studies of Malim, et al[10]. Second, once an APOBEC3G-loaded virus particle enters a susceptible target cell, reverse transcription will occur and APOBEC3G will then have an opportunity to deaminate nascent cytosines in the retroviral cDNA. The second prediction was particularly crucial because C → U deamination events could directly explain both the diminished infectivity of HIV ΔVif particles and the accumulation of strand-specific C/G → T/A transition mutations detected in patients.

Several groups simultaneously provided evidence in favor of such a cDNA deamination mechanism[23-26]. The HIV ΔVif (or murine leukemia virus) produced in the presence of APOBEC3G showed dramatically decreased levels of infectivity, and the resulting proviral DNA contained extremely high levels of plus-strand G → A transition mutations. In comparison, HIV ΔVif particles produced in the absence of APOBEC3G had normal infectivity, and the resulting proviral DNA harbored only a few non-strand, biased base substitutions. Biochemical studies with purified APOBEC3G confirmed that it preferentially deaminated cytosines within single-stranded DNA, but not within RNA, double-strand DNA, or RNA-DNA hybrid[23,27-30].

Of course a cDNA cytosine deamination mechanism means that APOBEC3G must somehow access the core of a newly produced HIV ΔVif particle. Several of the original studies verified that APOBEC3G could be incorporated into virus particles[10,23,25]. Subsequent work demonstrated that the incorporation of APOBEC3G is dependent on a specific interaction with the nucleocapsid region of the virus' group-specific antigen (Gag) protein[31-36].

However, there is some debate in this area, as a subset of these studies has also shown that the APOBEC3G-Gag interaction is dependent on RNA[35-37]. An RNA requirement or an RNA bridge is indeed plausible because both Gag and APOBEC3G have been shown to bind RNA[38,39]. Thus, although it is clear that APOBEC3G incorporates via Gag, biochemical and structural studies will be required to reveal the precise atomic interactions between these two proteins.

Interestingly, viral cDNA cytosine deamination may not be the only mechanism by which APOBEC3G inhibits HIV infectivity. APOBEC3G has two, conserved, zinc-binding cytosine deaminase motifs, one located within the N-terminal half and another within the C-terminal half of the protein. Several groups have shown that only the C-terminal deaminase catalyzes DNA cytosine deamination[40-42]. However, APOBEC3G catalytic mutants have been shown to retain strong anti-HIV activity[40,43]. Only when both the N- and the C-terminal

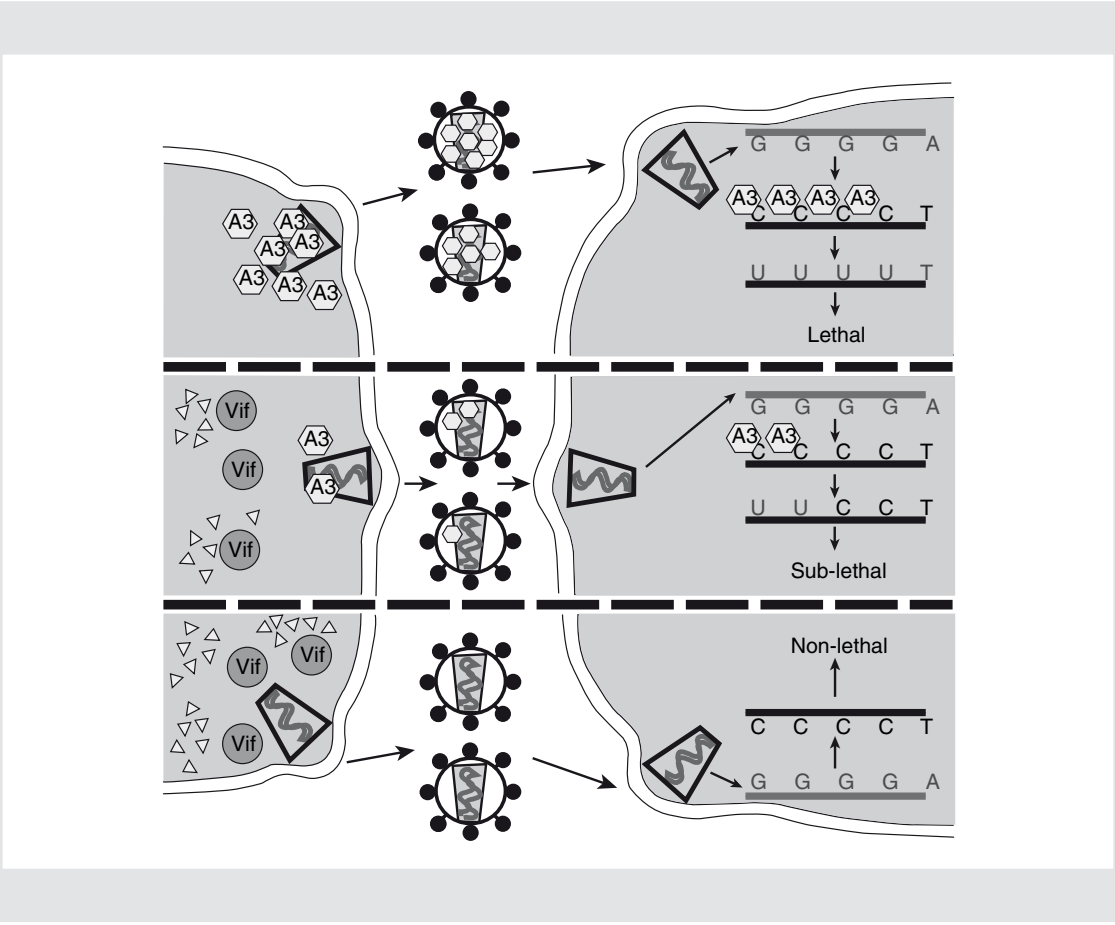

Figure 1. *Model for HIV restriction by APOBEC3 proteins. The upper panel illustrates virus production and infection in the absence of Vif. When Vif is absent, APOBEC3 proteins incorporate into viral particles and, upon infection of a new cell, deaminate cytosines to uracils in the first strand cDNA during reverse transcription. Such mutations are called "lethal" because they occur in such high numbers that the resulting provirus is so heavily mutated that it is rendered noninfectious. The middle panel illustrates a plausible scenario in vivo. In the presence of Vif, there may be instances in which a few APOBEC3 molecules escape Vif, incorporate into the viral particle, and trigger sub-lethal levels of mutations. Some of these mutations may be beneficial, such that they may promote virus evolution and escape from immune responses and anti-HIV drugs. The bottom panel illustrates the situation where Vif always wins the battle. In such case, no APOBEC3 molecules incorporate into viral particles and therefore the provirus escapes APOBEC3 lesions.*

deaminase domains were inactivated by amino acid substitutions was APOBEC3G rendered ineffective.

Thus, APOBEC3G appears to inhibit HIV infection through both deaminase-dependent and -independent mechanisms. Details of the deaminase-independent mechanism have yet to be revealed, and the relative contribution of each mechanism to HIV restriction remains to be determined.

HIV restriction by other APOBEC3 proteins

The ability of APOBEC3G to restrict HIV ΔVif and the high degree of similarity between it and other human nucleic acid cytosine deaminase family members prompted investigators to ask whether other APOBEC could similarly inhibit HIV (Table 1). Of all APOBEC family members tested, APOBEC3B, APOBEC3C, and APOBEC3F were able to restrict the infectivity of HIV ΔVif. Parallel controlled experiments indicated that the relative antiretroviral activities rank as follows: APOBEC3G > APOBEC3F > APOBEC3B > APOBEC3C (note that APOBEC3C was at best a weak inhibitor)[41,44-48]. None of the human APOBEC family members outside the immediate APOBEC3 family (APOBEC1, APOBEC2) or activation-induced cytidine deaminase, could diminish HIV infection[44,46,47].

With four different APOBEC3 proteins showing anti-HIV activity, it is important to ask which is active *in vivo*? Although this question has not been answered definitively, three clues help organize the candidates.

First, patient-derived, hypermutated, HIV-1 DNA sequences have shown G → A transitions within both 5'-GG

Table 1. The human APOBEC family members and their editing and anti-HIV activities

Protein	Editing activities	HIV restriction	References
AID	DNA deaminase	No	18,44,47
APOBEC1	DNA or RNA deaminase	No†	12,18,44,47
APOBEC2	Unknown	No	47,91
APOBEC3A	DNA deaminase	No	44,46,92,93
APOBEC3B	DNA deaminase (with possible RNA editing activity*)	Yes	44,48
APOBEC3C	DNA deaminase	Yes	18,41
APOBEC3D	Unknown	Unknown	18
APOBEC3D-3E	None	No	18,94
APOBEC3E	Unknown	Unknown	18
APOBEC3F	DNA deaminase (with possible RNA editing activity*)	Yes	44-47
APOBEC3G	DNA deaminase	Yes	10,18,23-26
APOBEC3H	DNA deaminase	No†	95
APOBEC4	Unknown	Unknown	96

*A low level of plus-strand C → T transition mutations has been detected in proviral DNA resulting from HIV particles produced in the presence of APOBEC3B or APOBEC3F[44,45]; plus-strand C → T = RNA or dsDNA editing; plus-strand G → A = cDNA editing.
†Rat APOBEC1 can restrict HIV-1 ΔVif[44]; African green monkey (AGM) APOBEC3H can restrict SIVAGM ΔVif[95].
AID: activation-induced cytidine deaminase.

and 5'-GA dinucleotide sequences (5'-CC and 5'-TC on the minus strand, with the mutated site underlined[19,21,22]). Only APOBEC3G has been shown to predominantly cause G → A transitions within the 5'-GG dinucleotide sequence[23,25,26], strongly indicating that this protein is important *in vivo.* In contrast, APOBEC3B, -3C, and -3F all prefer to deaminate 5'-TC dinucleotides, which manifest as plus strand 5'-GA → -AA hypermutations[41,44-46,48]. Local mutation signatures, therefore, cannot be used exclusively to implicate one of these.

Second, gene expression studies have indicated that APOBEC3F and APOBEC3G are expressed in a coordinated manner in a number of human tissues, including the primary sites where HIV replicates (CD4+ T-cells and macrophages)[45,46]. In contrast, APOBEC3B appeared to be expressed at barely detectable levels in these tissues.

Third, only APOBEC3F and APOBEC3G, but not the others, are significantly counteracted by HIV-1 Vif indicating that these are the most important *in vivo*, otherwise, why would HIV go through the trouble of inhibiting them? (see below).

These studies, together with APOBEC3F-APOBEC3G immuno-precipitation data[46], combined to suggest that APOBEC3F and APOBEC3G work together to mediate HIV-1 restriction *in vivo*; APOBEC3B and APOBEC3C may have only minor or negligible roles. Nevertheless, short of identifying APOBEC3 variants in the human population, and which are HIV-resistant or -susceptible, the question of which APOBEC3 are most important *in vivo* will be difficult to answer.

HIV survives by neutralizing APOBEC3 proteins

Obviously, HIV successfully counteracts the APOBEC3 protein(s) *in vivo*. Several independent observations suggested that Vif functions by preventing the incorporation of APOBEC3G.

First, careful measurements of APOBEC3G levels in purified virus particles indicated that very little APOBEC3G incorporates when Vif is present[49-52]. Second, cellular APOBEC3G levels were noticeably lower when Vif was co-expressed (even without other virus proteins)[49-53]. Fluorescent microscopy produced one of the most dramatic results because Vif expression clearly quenched GFP-tagged APOBEC3G[53]. These observations suggested that Vif prevented the incorporation of APOBEC3G by promoting its degradation (Fig. 1, bottom panel). Se-

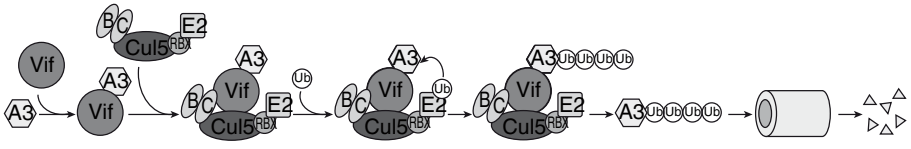

Figure 2. *The mechanism of Vif-dependent degradation of APOBEC3 proteins. Vif binds APOBEC3 (A3 in the figure), which forms a complex that recruits the cellular proteins ELONGINB/C (B and C in the figure). This complex then mediates CULLIN 5 (CUL5 in the figure) -RBX1-dependent ubiquitylation of APOBEC3 protein by the E2 ubiquitin-conjugating enzyme. Ubiquitylation of APOBEC3 proteins results in their degradation by the proteasome.*

veral groups have provided evidence in favor of such a mechanism, showing that the inclusion of proteasome inhibitors raised cellular (and viral) APOBEC3G levels despite the presence of Vif[50-55].

The precise mechanism used by Vif to promote APOBEC3G degradation crystallized when Yu, et al. reported components of a Vif-interacting complex[56]. An E3 ubiquitin ligase complex consisting of the cellular proteins CULLIN5, ELONGINB, ELONGINC, and RBX was implicated in the degradation of APOBEC3G[56]. The ability of Vif to neutralize APOBEC3G was shown to require the conserved amino acid motif SLQxLxL, which is found in the Vif proteins of all primate lentiviruses[57]. This motif is similar to that of other cellular and viral ELONGINB/C-binding proteins, and it was therefore called a BC-box[50,58-61]. The requirement for the BC-box strongly suggested that Vif interacts with the E3 ubiquitin ligation complex through ELONGINC. However, Vif was also shown to interact with CULLIN5 via a novel H-x_5-C-x_{17-18}-C-x_{3-5}-H zinc-binding motif, which is also conserved amongst primate lentiviral Vif proteins[60-62]. Mutating either of the histidines or the cysteines abolished this interaction and simultaneously disrupted the ability of Vif to neutralize APOBEC3G[60-62]. Moreover, residues 12-128 of Vif have been implicated in interacting with APOBEC3G[50]. These data have combined to indicate that Vif functions by nucleating an ubiquitin degradation complex, which includes APOBEC3G and the ubiquitin ligation factors CULLIN5, ELONGINB/C, and RBX (Fig. 2).

Does Vif neutralize other APOBEC3 proteins by a similar proteasome-dependent mechanism? Like its effect on APOBEC3G, expression of HIV Vif was shown to improve the infectivity of viruses produced in the presence of APOBEC3F[45-47,63] and it reduced both the viral and cellular levels of APOBEC3F[44-47,63]. The Vif-dependent degradation of APOBEC3F was also shown to occur through the proteasome and require CULLIN5, ELONGINB/C, and RBX[63]. In contrast, APOBEC3B and APOBEC3C appeared resistant to Vif [41,44,48,64]. These observations were further supported by experiments indicating that Vif could specifically interact with APOBEC3F, but not with APOBEC3B[48,63]. As discussed above, the discovery that Vif could neutralize APOBEC3F suggests that APOBEC3F may also function to inhibit HIV infectivity *in vivo.*

Incomplete neutralization of APOBEC3F and APOBEC3G *in vivo*?

Despite the remarkable ability of Vif to counteract APOBEC3F and APOBEC3G, some evidence has accumulated which indicates that Vif is not completely effective *in vivo.* Many reports have detected G → A hypermutation from HIV proviral sequences obtained from infected individuals[19-22,65]. Such G → A hypermutation is predominantly found within 5'-GA and 5'-GG sequences[19,21,22], reflecting the dinucleotide preferences of APOBEC3F and APOBEC3G, respectively[23,25,26,44-46]. Interestingly, mutations within the APOBEC3F consensus have been found nearly twice as often as those within the APOBEC3G consensus[19,22]. This may be explained by the fact that APOBEC3F is more resistant to Vif [45]. However, it should be noted that these data were obtained under specific laboratory conditions with a single Vif-APOBEC3F combination. Given the extreme variability in HIV-1 Vif sequences *in vivo,* other variants may have different outcomes.

Indeed, Simon, et al. provided additional evidence suggesting that both APOBEC3F and APOBEC3G might at times be incompletely blocked *in vivo*[66]. This study analyzed the anti-APOBEC3 activity of many naturally occurring Vif variants. It revealed that partially and completely defective *vif* alleles are surprisingly prevalent in viral isolates recovered from HIV-positive individuals. Interestingly, many of the Vif variants were unable or only partially able to neutralize APOBEC3F, APOBEC3G, or both proteins. Moreover, some of the HIV-infected individuals carrying fully or partially defective Vif alleles showed a prevalence of HIV proviral DNA sequences with G → A

hypermutation attributable to APOBEC3F and/or APOBEC3G. However, it is important to note that all of the aforementioned hypermutation studies may have examined lethally mutated proviral DNA (integrated), and they therefore leave open the question of whether the majority of the hypermutation HIV sequences detected *in vivo* represent "dead-end", fully-restricted virus pools.

Thus far we have presented evidence that APOBEC3G and APOBEC3F can lethally mutate HIV ΔVif, and data that HIV Vif can effectively disarm the cellular defense by triggering the degradation of these DNA cytosine deaminases (Fig. 1, top and bottom panels, respectively). However, within an infected individual a delicate balance likely exists between the activities of the APOBEC3 proteins and Vif, such that minor disturbances may strongly influence the outcome of an infection. We hypothesize that within infected individuals there will be instances in which one or more APOBEC3 molecules are able to incorporate into budding virions, gain access to the viral genome, and trigger sub-lethal levels of G → A mutation (Fig. 1, middle panel). Some of these mutations are likely to compromise the fitness of the virus. However, many of these mutations may be beneficial as they may very well help the virus directly evade the immune response or become drug resistant.

The HIV drug-resistance problem

Highly active antiretroviral therapy (HAART), which consists of three or more drugs that inhibit HIV reverse transcriptase or protease, has remarkably improved the prognosis of individuals infected with HIV[67]. HAART is capable of suppressing viral replication to virtually undetectable levels (< 50 HIV genomic RNA copies per milliliter of blood)[68]. However, suppressing HIV replication with HAART is a lifelong challenge, as viral loads quickly rebound in the absence of drug treatment (within 2-12 weeks), and it has been estimated that it would take about 70 years of therapy to fully eradicate all HIV-infected cells within an individual[69,70]. Moreover, insufficient cellular drug uptake, high rates of drug metabolism, or incomplete adherence to medication can all contribute to inadequate drug concentrations, which in turn can lead to an incomplete suppression of viral replication[71]. A low level of viral replication increases the chance of a drug-resistant HIV variant emerging. In many but not all instances of HAART failure, HIV has developed resistance to all of the components of the cocktail[72,73]. The problem of drug resistance is even greater (and nearly inevitable) in many underdeveloped regions of the world where anti-HIV resources are limited and only single-drug therapies are used in an attempt to treat infections[74].

The origin of drug-resistant HIV variants

It is widely believed that many HIV drug-resistance-conferring mutations preexist in the patient's viral population[75,76]. The highly error-prone HIV reverse transcriptase is considered the major source of viral mutations. The *in vivo* mutation rate for HIV-1 was determined to be 4 x 10^{-5} mutations per base pair per replication cycle, which predicts that about one mutation occurs for every three new genomes produced[77,78]. Moreover, some drugs even exacerbate mutation rates, possibly by decreasing the replicative fidelity of the viral proteins[79,80].

High mutation rates and massive levels of replication (estimated to be about 10^{10} to 10^{12} new virions per day[81]) result in the generation of a large pool of HIV variants. Thus, HAART and especially single-drug therapies can readily select for the emergence of drug-resistant variants from within this large population of virus variants.

As discussed earlier, Vif does not always inhibit the APOBEC3 proteins *in vivo.* This is primarily evidenced by the fact that G → A hypermutations are detected readily in HIV proviral sequences obtained from patients[22]. However, as already alluded to, all current hypermutation studies suggesting that the APOBEC3 proteins contribute to HIV variation *in vivo* may have exclusively sampled "dead-end" populations of proviruses, which were lethally mutated by the APOBEC3 proteins. Sequences representing live, replication-competent viruses may not have been examined.

There are, however, additional substantial hints suggesting that the APOBEC3 proteins contribute to HIV variation *in vivo,* and that the conflict between the APOBEC3 proteins and the ancestors of HIV (presumably the simian lentiviruses) has been ongoing for some time. First, the HIV and SIV genomes have an extreme plus-strand adenine bias (± 40%), largely at the expense of plus-strand guanines (± 23%). This contrasts directly with the coding-strand adenine and guanine contents of their host (estimated to be ± 25%). Second, as discussed previously, APOBEC3G shows a DNA deamination preference for 5'-C<u>C</u> dinucleotides (5'<u>G</u>G on the plus-strand). This DNA preference can be further extended to 5'-C<u>G</u>GG or 5'-T<u>G</u>GG, and interestingly, only 5'-C<u>G</u>GG (but not 5'-T<u>G</u>GG) is strongly under-represented in the HIV genome[82]. The CGG encodes the amino acid arginine, which can be encoded by an additional five codons, CGN and AG(W) (W = G or A). The HIV uses CGG very infrequently (3%), but shows a strikingly strong bias for

AGA (65%), which is not an APOBEC3G DNA hotspot[82,83]. On the other hand, TGG is the only codon for tryptophan (Trp) and, therefore, TGG cannot be selected against without loosing the ability to encode Trp. This perhaps explains why 5'-CGGG (not 5'-TGGG) is strongly selected against, even though it is a DNA site that is clearly preferred by APOBEC3G. These observations, and in particular the A-richness of the HIV and SIV genomes, imply that the APOBEC3 proteins have been shaping SIV genomes, and more recently HIV genomes, for ages.

Virus evolution is a relatively complex biologic process to study. However, drug-resistance selections have been used to begin to understand how the virus can adapt to selective forces. There are several clear examples in the literature suggesting that APOBEC3 proteins can contribute to HIV drug resistance, as a number of important mutations associated with resistance occur at APOBEC3F or APOBEC3G hotspots, 5'-GA → -AA and 5'-GG → -AG, respectively. Here we will discuss only a few of the most prominent examples.

First, nelfinavir is a common component of HAART, and it functions by inhibiting the HIV protease[84]. Three of the most common mutations in protease that are associated with nelfinavir resistance (D30N, M36I and M46I) are G → A transition mutations that map to APOBEC3F hotspots, 5'-GA → 5'-AA[84,85] (Fig. 3).

Second, the thymidine analog zidovudine is another common component of HAART, and it works by inhibiting the reverse transcriptase enzyme[86]. Amino acid substitutions that confer resistance to zidovudine are called thymidine analog mutations, and they include M41L, D67N, K70R, L210W, T215F/Y, and K219Q/E/N/R[87]. Interestingly, D67N can clearly be caused by a G → A nucleotide change mapping to an APOBEC3F hotspot (Fig. 3).

Third, lamivudine is another reverse transcriptase inhibitor and common component of HAART. Contrary to the other anti-HIV drugs, resistance to lamivudine emerges when reverse transcriptase acquires one single mutation at amino acid M184[86,88]. Both the M184I and M184V substitutions confer high-level resistance to lamivudine. Interestingly, the M184I substitution is a G → A transition mutation that maps to an APOBEC3G hotspot, 5'-GG → 5'-AG (Fig. 3).

Of course, simply because a particular drug-resistance mutation maps to an APOBEC3F or APOBEC3G mutational hotspot does not necessarily indicate that the APOBEC3 protein actually created that lesion. For instance, purified HIV reverse transcriptase can cause G → A transition mutations in 5'-GG and 5'-GA dinucleotides, particularly under biased dNTP pool concentrations[89]. Nevertheless, the observation that many drug-resistance mutations are located within APOBEC3 DNA hotspots is very intriguing. The possibility that APOBEC3 proteins impact HIV drug resistance needs to be addressed by well-controlled experiments.

In a recent report, Berkhout, et al. speculated on the possible role of APOBEC3 proteins in HIV evolution and drug resistance[90]. In this report, the authors tabulated all mutations associated with HIV drug resistance. Interestingly, this survey revealed that G → A transitions are the most common nucleotide changes associated with drug resistance with a frequency of 22%. Such a preference toward G → A supports a role for APOBEC3 proteins in HIV drug resistance. However, Berkhout, et al. argued that a large number of G → A changes are found in viruses produced from APOBEC3G-negative cells and, therefore, the idea that reverse transcriptase errors are more important in the development of HIV drug resistance was favored.

The aforementioned observations are compatible with a model for HIV restriction in which the APOBEC3 proteins contribute to the generation of HIV genetic variation (Fig. 1, middle panel). In many cells, small amounts of APOBEC3 protein may escape Vif, enter viral particles, and trigger "sub-lethal" levels of mutation. Whether such sub-lethal mutations contribute to HIV sequence diversification, and therefore also to immune escape, evolution and drug resistance, remains an open question.

Concluding remarks

There is clear evidence that APOBEC3 proteins restrict retroviral infection and represent a novel form of cellular innate immunity. AIDS remains a problem in part because Vif-expressing HIV can usually fend off the onslaught of APOBEC3 proteins. However, other observations suggest that APOBEC3 expression levels in cells may not, in some contexts, be adequate to extinguish viral infectivity. Such sub-lethal levels of APOBEC3 expression opens a window of opportunity for HIV to either completely escape the lethal mutagenic effects of APOBEC3 proteins or, and more intriguingly, perhaps acquire sub-lethal mutations that subsequently impact such properties as virus fitness, drug resistance, immune evasion, and virus evolution.

Taking the example of HIV drug resistance, there is no definitive experimental support, but rather only circumstantial evidence, that argues in favor of the APOBEC3 proteins contributing to these phenotypes. Nonetheless, the possibility that many resistance-conferring mutations may be attributable to the DNA cytosine deaminase activity of APOBEC3 proteins leaves open

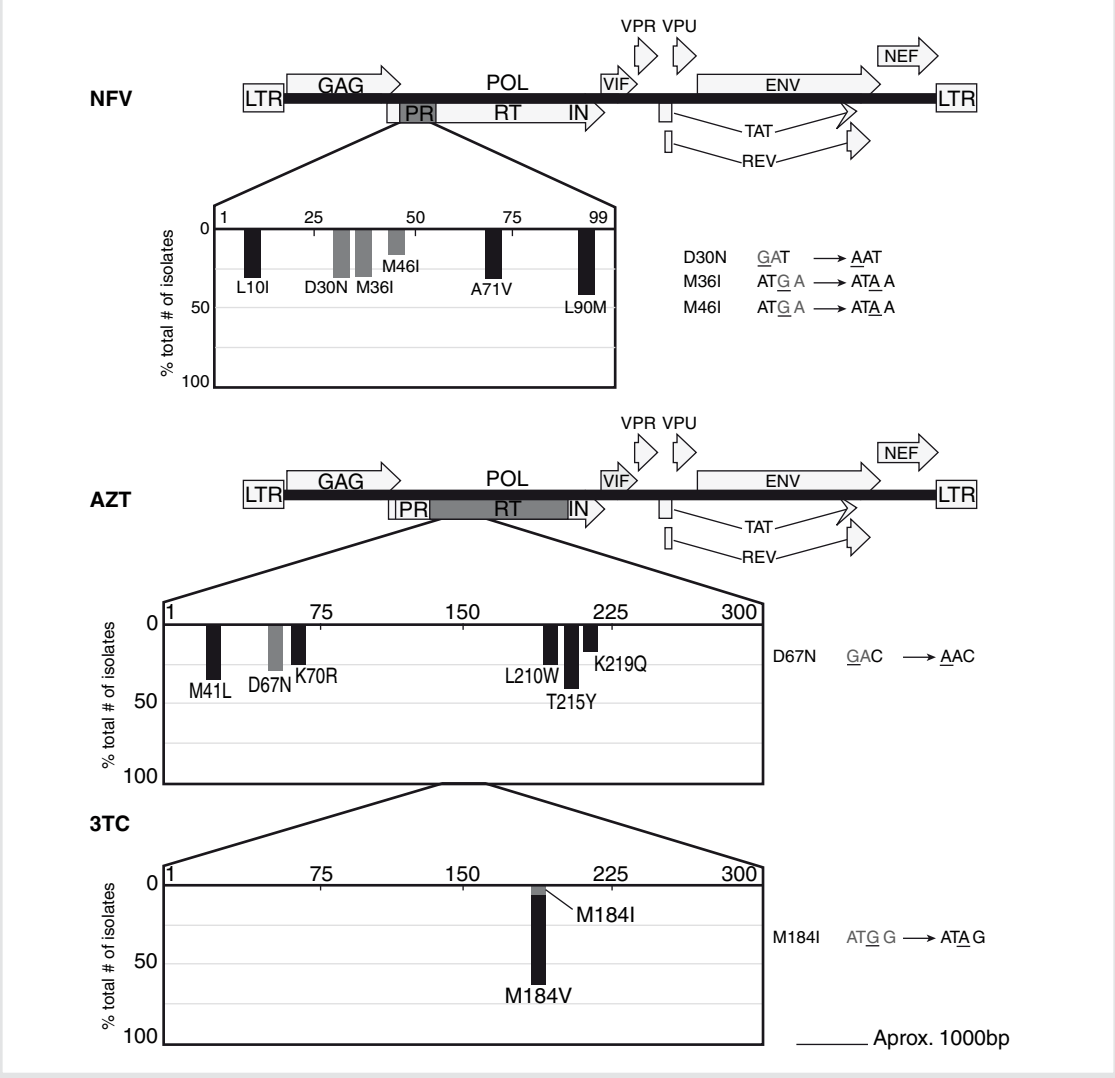

Figure 3. *Sites of common HIV drug resistance mutations. A schematic of the HIV-1 genome showing sites of common drug-resistance mutations and their frequencies in drug-treated subjects. Mutation frequencies were obtained using the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu/index.html). Red bars represent the frequencies of drug-resistance mutations that map to APOBEC3F or APOBEC3G hotspots. Black bars represent the frequencies of drug-resistance mutations that do not map to APOBEC3F or APOBEC3G hotspots. NFV: nelfinavir; AZT: azidothymidine; 3TC: lamivudine.*

the opportunity for further experimentation directed at this question.

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