APOBEC3 Cytidine Deaminases: Distinct Antiviral Actions along the Retroviral Life Cycle*

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The field of human immunodeficiency virus (HIV) biology has been galvanized by the discovery of innate APOBEC3 cytidine deaminases, which pose powerful barriers to the replication of HIV and other retroviruses. Rapid progress has been made in defining their action, intriguing regulation within cells, expanded range of retroviral targets, and counterstrategies utilized by retroviruses against them. Although scientifically fascinating, advances in APOBEC3 biology may lead to new antiviral drugs and improved lentiviral vectors for gene therapy.

Discovery of APOBEC3G

Primate lentiviruses, except equine infectious anemia virus, encode a viral infectivity factor (Vif) protein. HIV-1 Vif is a 23-kDa basic phosphoprotein expressed late in the retroviral life cycle. Viruses deficient in vif (Δvif) cannot mount a spreading infection when produced in "nonpermissive" cells, such as primary human CD4 T cells and macrophages, the primary targets of HIV-1. Conversely, many "permissive" laboratory-adapted T cell lines support HIV-1 infection without Vif (1, 2). The nonpermissive phenotype proved dominant in heterokaryons formed by fusing these two types of cells (3, 4). Thus, nonpermissive cells selectively express an antiviral activity that is overcome by Vif. This Vif-targeted antifactor was identified as APOBEC3G (A3G) (5), which is effectively encased in budding virions only in the absence of Vif (6, 7).

APOBEC Cytidine Deaminases

A3G belongs to a large family of tissue-specific cytidine deaminases (8) that display RNA editing and/or DNA mutator activity (9, 10). APOBEC1 (9) and activation-induced deaminase (11) are tandemly arranged on chromosome 12. In intestinal epithelial cells, APOBEC1 is the key component of an RNA editosome complex (12) that deaminates cytosine 6666 in mammalian apolipoprotein B mRNA, resulting in a truncated version of apoB. In germinal center B cells, activation-induced deaminase is required for class-switch recombination and somatic hypermutation (11). Both enzymes catalyze deamination of dC residues in single-stranded DNA in vitro (10, 13, 14). Remarkably, activation-induced deaminase has no measurable deaminase activity unless first pretreated with RNase to remove small inhibitory RNAs bound to the enzyme (15). Uncontrolled activity of APOBEC1, activation-induced deaminase, and perhaps other cytidine deaminases can transform cells (16, 17). Thus, they may be subject to intracellular regulation to curtail such events.

A3G is one of a cluster of related genes found on chromosome 22 (8). This APOBEC3 locus shows evidence of expansion by tandem duplication and unequal crossover after the genetic radiation of mice and humans. All APOBEC3 genes (A3A–A3H) are arranged in the same orientation. Their pattern of expression is tissue-specific. A3G, -3F, and -3C are expressed in spleen, peripheral blood lymphocytes, ovary, and testes, whereas little or no A3B mRNA is detectable in these tissues (8, 18). However, A3B, A3C, and to a lesser extent A3A mRNAs are prevalent in various cancer cell lines (8). Active APOBEC3 gene products function as homodimers although heterodimers can occur. The enzyme active site contains conserved zinc ligands (C/H)X(E and PCXXC. These proteins also contain a key glutamate required for protein shuttling during catalysis and two key aromatic residues involved in RNA substrate binding (8).

Mutagenesis by Virion-incorporated APOBEC3G

A3G packaging into HIV-1 virions involves assembly with the nucleocapsid region of the Gag viral protein and possibly binding of RNA (19–26). A3G appears to selectively target single-stranded DNA (27, 28) and yields dC to dU mutations in the viral minus-strand DNA formed during reverse transcription (RT) (5, 29–32). These uracil-containing DNA molecules are either degraded, probably by DNA repair enzymes such as uracil DNA glycosylase (33) and apurinic-apyrimidinic endonucleases, or serve as templates for the synthesis of plus-strand DNA, yielding dG to dA hypermutated proviruses encoding altered proteins. These events potently halt viral spread. The single-stranded specificity also accounts for the increase in mutation frequency in a 5' to 3' direction (27). The 3' regions in the minus-strand remain single-stranded for the longest time as they await plus-strand synthesis. This antiviral activity of human A3G also extends to other lentiviruses including simian immunodeficiency virus (SIV), equine infectious anemia virus (EIAV), and even distantly related retroviruses such as murine leukemia virus (MLV) and foamy viruses (6, 9, 31, 34, 35).

Other Antiviral APOBEC3 Proteins

Strikingly, other APOBEC3 family members also exert antiviral activity. A3F induces dG to dA transitions in viral plus-strand DNAs, and its action is blocked by HIV-1 Vif (18, 36–38). However, the two enzymes differ in their consensus sequence for mutation: A3G favors 5'-CC dinucleotides (underline denotes the site of deamination) (27, 29, 31, 32), whereas A3F prefers 5'-TTG (18, 36, 37). Mutations in the A/T-rich proviruses present in AIDS patients bear the footprints of both A3G and A3F action. The hypermutation specificity of A3F appears to be governed by the C-terminal deaminase domain (39). Human A3B also has moderate activity against HIV-1 and an A3F-like target site consensus. However, A3B is resistant to Vif (18, 40). A3B is not expressed in many of the natural cellular targets of HIV-1 and may not normally influence HIV-1 replication in vivo. Neither A3F nor A3B is active against MLV whereas A3G is (18, 41). Thus, the spectrum of viruses impaired by A3F and A3B may well be more restricted.

Vif Neutralizes APOBEC3 Proteins

Lentiviruses have evolved a remarkable ability to neutralize A3G. Coexpression of HIV-1 Vif and A3G in transiently transfected human or monkey cell cultures significantly decreases A3G protein expression (6, 7, 42–47). Importantly, this reduction also occurs in HIV-1-infected T cells (7), making this antiviral enzyme unavailable for subsequent incorporation into progeny virions. Thus, the antiviral effect of A3G is forfeited.

Vif appears to act by accelerating degradation of A3G by the 26 S proteasome and partially impairing translation of A3G mRNA (7). Vif induces polyubiquitylation of A3G and targets it for destruction by the proteasome (45, 46). Vif contains two key domains, one that binds to A3G and a SLQ(Y/F)/LA motif for A3G degradation via the proteasome (45). The latter is similar to a conserved sequence in the BC-box of the suppressors of cytokine signaling proteins. Vif recruits an E3 ligase complex of elongins B and C, Cul5, and Rbx1 (47). Binding of Vif to elongin C depends on the SLQ(Y/F)/LA motif in Vif and two conserved cysteines (48) and is negatively regulated by serine phosphorylation in the BC-box motif (49). Dominant-negative mutants of Cul5, which block modification by Nedd8 or assembly with Rbx1, prevent the degradation of A3G and restore its antiviral activity (47). Vif also induces proteasomal degradation of A3F (36–38, 50).

Unlike A3G, the viral protein conferred by Vif appears highly species-
specific (6). For example, although Vif from the SIV of African green monkey (SIVagm) triggers the degradation of its own host’s A3G, it fails to neutralize human or chimpanzee A3G. Similarly, HIV-1 Vif does not impair the action of African green monkey or rhesus macaque A3G. These species-specific effects are likely key to the species tropisms of retroviruses and may thwart many cross-species transmissions. Remarkably, this apparent specificity is regulated by amino acid 128 in A3G (aspartic acid in humans and lysine in African green monkey) (51–54). Interestingly, Vif of SIV from rhesus macaque (SIVmac), and possibly sooty mangabey (SIVsm), neutralizes A3G orthologs in most of the other species, including humans (6, 54), a compelling correlation with the fact that SIVsm was zoonically transmitted to humans and gave rise to HIV-2.

Although available data argue that Vif-induced degradation of A3G is key. Vif might also physically exclude A3G from virions by sequestering it from sites of viral budding (6, 42). Moreover, the intracellular depletion of A3G and rescue of viral infectivity might be functionally separable activities of Vif (49, 55). For example, infectious HIV viruses can be produced by cells where Vif only moderately alters the steady-state A3G levels (55). The S144A mutation in Vif, which prevents phosphorylation at this site, compromises infectivity of progeny virions but induces A3G degradation (49). Thus, Vif phosphorylation may regulate another Vif function that confers full viral infectivity. Such ancillary actions of Vif deserve continued investigation.

Counterstrokes by Other Susceptible Viruses

Other retroviruses have evolved different escape mechanisms. Several APOBEC3 proteins potently inhibit the infectivity of viral vectors based on primate foamy viruses (PFV). The packaging of APOBEC3 into PFV virions induces dG to dA deamination in PFV reverse transcripts. PFV Vif appears to function as the foamy virus analogue of HIV-1 Vif preventing the incorporation of the human A3G/A3F proteins into progeny virions. However, Bet does not deplete human A3G/A3F in virion producer cells (35). Similar findings have emerged in studies of a distantly related feline foamy virus (34). Another example is provided by MLV, which replicates effectively in murine APOBEC3-positive cells yet does not encode a vif- or a bet-like gene. The MLV Gag protein has evolved to lack binding, and hence virion packaging, of the cognate murine APOBEC3. However, MLV infectivity is still restricted by heterologous APOBEC3 proteins that retain the ability to interact with MLV Gag (41, 56). The lentivirus EIAV, also devoid of a Vif but sensitive to human A3G, may have evolved another mechanism for evading A3G orthologs in its natural hosts. It may replicate in cells lacking the enzyme, but an occult vif-like activity or a Gag protein intrinsic to the deaminase cannot be excluded. EIAV, similar to feline immunodeficiency virus, also encodes a dUTPase that may provide a separate pathway to protect the virus from the effects of deamination.

A3G/A3F appears to block the incorporation of HBV RNA into subgenomic particles, destabilize the viral RT complex, and preclude HBV DNA accumulation in virions. Cytidine deaminase activity seems dispensable (64). The same phenomenon prevails in the HepG2 hepatoma cell line, but APOBEC3-mediated editing occurs in some HBV DNAAs (66–69). Although the precise antiviral mechanism requires further investigation, it seems to parallel the post-entry restricting function of A3G against HIV, which may not be strictly dependent on cytidine deamination.

The deaminase-independent antiviral activity of A3G also targets HTLV-1 (70). A3G packaged into HTLV-1 particles can reduce infectivity of HTLV-1 particles (70) although the effects were more modest when compared with HIV-1 (71). Signature mutations, however, do not accumulate at significant levels (70). HTLV-1 preferentially infects activated CD4 T cells that express key receptors for viral entry and thus may bypass the post-entry restricting activity of LMM A3G in resting CD4 T cells.

Roles beyond Blocking Exogenous Retroelements

A3G might also act on endogenous retroelements. These elements, which correspond to long interspersed nuclear elements (LINEs), short interspersed nuclear elements, and long terminal repeat (LTR) retrotransposons, are present at high copy number in ancestral genomes and have contributed as a major driving force to genome evolution (72). Most retroelements have lost replication competence, but some, including murine intracisternal A-particle and MusD sequences, remain mobile through retrotransposition, an intracellular process involving RT intermediates. Both human and murine A3Gs markedly inhibit retrotransposition of intracisternal A-particle and MusD elements (73) and even the yeast Ty1 LTR retrotransposon (74, 75) in vitro. Cytidine deaminase activity appears critical for this inhibition (73, 75). Indeed, the mouse genome contains many retrotransposon proviruses that harbor mutations consistent with APOBEC3-mediated editing (73). In addition, APOBEC genes have been subject to strong positive selection throughout the history of pri- mate evolution (76, 77). This selection appears older than the modern lentiviruses, suggesting that these genes expanded to prevent genome instability caused by endogenous retroviruses (77). Surprisingly, LINE-1 (L1) non-LTR retrotransposons do not seem to be affected by A3G (73, 78). This could reflect the nuclear localization of its RT intermediates. Nonetheless, the effect of APOBEC3 proteins on non-LTR retroelements requires careful evaluation. Their replication cycle involves cytoplasmic RNA intermediates, and the APOBEC3 proteins are competent for RNA binding (8). Furthermore, the relatively narrow tissue distribution of the APOBEC3 proteins in immune cells and germ line cells (8) raises the intriguing possibility of a natural editing substrate essential for the function of these cells or the presence of harmful elements in these cells that must be nullified. Significant effort should be aimed at identifying the “natural” cellular targets of APOBEC3 proteins.
APOBEC3 as New Therapeutic Targets

Insights into the mechanism by which Vif counteracts A3G have revealed promising new targets for anti-HIV-1 drug development. The optimal strategy would be to identify small molecule inhibitors that selectively disrupt the association of Vif with A3G/A3F proteins. Others might include blocking recruitment of the E3 ligase by Vif. By suppressing the activity of Vif, these drugs would free the endogenous A3G/A3F proteins to act against HIV-1.

Information on factors that regulate APOBEC3 expression might also provide a means to counteract the Vif-mediated degradation. A3G is at low levels in resting T cells but strongly induced when the cells are activated (7). PMA stimulation of H9 T cells activates A3G gene transcription involving the mitogen-activated protein kinase signaling pathway (79). Identifying the signaling pathways involved in induction of APOBEC3 expression might be therapeutically valuable if APOBEC3 levels could be safely boosted beyond the capacity of Vif.

The discovery that cellular LMM A3G is a post-entry restriction factor in resting CD4 T cells (57) could prompt evaluation of approaches to produce this restricting activity or prevent its natural inactivation in cells that are normally highly permissive for HIV-1 infection. The conversion of LMM to HMM A3G after T cell activation merits careful assessment regarding the range of signals and the molecular pathways capable of inducing this response. Cytokines may confer permissivity for HIV-1 infection in resting T cells (80, 81). Thus, cytokine stimulation could promote the assembly of A3G into the latent HMM complexes as they create a permissive state for HIV-1 in resting T cells in the absence of full-fledged cellular activation and proliferation. It will be important to determine whether cytokine treatment of T cells can also up-regulate expression of A3G and whether the increasing A3G levels are required for the assembly of HMM complexes.

Another important task is to define the protein and RNA cofactors in the HMM A3G complex (57). It remains unclear whether these HMM complexes correspond to the A3G-containing cytoplasmic bodies that lack vimentin cages (82). Small molecule inhibitors of HMM A3G complex formation might be valuable, as this host protein-protein interface would not be expected to undergo rapid sequence variation like Vif. A putative mutant of A3G constitutively residing in the LMM complex, even upon T cell stimulation, would also provide early resistance against incoming viruses. However, an effect of such disassembled forms of A3G on host genomic DNA must be excluded in these activated and often proliferating cellular hosts. It will be of great value to determine if LMM but catalytically inactive A3G could mediate the post-entry block. For lentivirus vector-mediated gene therapy in T cells, stimuli promoting the HMM A3G complex formation without potentially promoting cell activation or differentiation could be a crucial step in developing therapeutic strategies for a variety of diseases including cancers.

The role of APOBEC3 members in HBV infection is unclear. APOBEC3 expression has not been demonstrated in human hepatocytes, the primary target for HBV. Interferons inhibit HBV replication by mediating noncytopathic HBV clearance in vivo and in liver cell lines (83). A specific APOBEC family member might be up-regulated in hepatocytes by interferons and in turn facilitate HBV clearance. A better understanding of the potential participation of APOBEC3 in this response could facilitate the identification of new therapeutic strategies for HBV.

Concluding Remarks

HIV-1 RT is vulnerable to lethal editing by virion-incorporated host A3G cytidine deaminase. Unfortunately, the HIV-1 Vif protein circumvents this potent antiviral defense. HIV-1 also encounters a powerful post-entry restriction block mediated by the LMM form of A3G present in resting CD4 T cells.
MINIREVIEW: APOBEC3G and HIV Defense

This new restricting activity of A3G is not strictly dependent on editing and is not antagonized by Vif. These findings highlight the multifunctional properties of A3G as an anti-factor acting at distinct steps along the retroviral life cycle, utilizing its inherent deaminase activity or a deaminase-independent antiviral mechanism. Acting in concert with other family members, A3G proteins also likely function as important guardians of the integrity of the human genome through their effects on endogenous retroviruses and possibly other retroelements.

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