Reassessing APOBEC3G Inhibition by HIV-1 Vif-Derived Peptides

Christopher M. Richards [1,2], Ming Li [1,2], Angela L. Perkins [3], Anurag Rathore [1], Daniel A. Harki [3] and Reuben S. Harris [1,2,4]

1 - Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 321 Church Street S.E., Minneapolis, MN 55455, USA
2 - Institute for Molecular Virology, University of Minnesota, 515 Delaware Street S.E., Minneapolis, MN 55455, USA
3 - Department of Medicinal Chemistry, University of Minnesota, 2231 6th St. S.E., Minneapolis, MN 55455, USA
4 - Howard Hughes Medical Institute, University of Minnesota, 2231 6th St. S.E., Minneapolis, MN 55455, USA

Correspondence to Reuben S. Harris: Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 321 Church Street S.E., Minneapolis, MN 55455, USA. rsh@umn.edu
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Abstract

The human APOBEC3G (A3G) enzyme restricts HIV-1 in the absence of the viral accessory protein viral infectivity factor (Vif) by deaminating viral cDNA cytosines to uracils. These uracil lesions base-pair with adenines during the completion of reverse transcription and result in A3G signature G-to-A mutations in the viral genome. Vif protects HIV-1 from A3G-mediated restriction by forming an E3-ubiquitin ligase complex to polyubiquitinate A3G and trigger its degradation. Prior studies indicated that Vif may also directly block the enzymatic activity of A3G and, provocatively, that Vif-derived peptides, Vif 25–39 and Vif 105–119, are similarly inhibitory. Here, we show that Vif 25–39 does not inhibit A3G enzymatic activity and that the inhibitory effect of Vif 105–119 and that of a shorter derivative Vif 107–115, although recapitulated, are non-specific. We also elaborate a simple method for assaying DNA cytosine deaminase activity that eliminates potential polymerase chain reaction-induced biases. Our results show that these Vif-derived peptides are unlikely to be useful as tools to study A3G function or as leads for the development of future therapeutics.

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Introduction

APOBEC3G (A3G) is a human innate immune effector enzyme with demonstrated potent activity against a wide variety of exogenous and endogenous parasitic elements (reviewed by Refs. [1–4]). Most studies have focused on the mechanism of A3G-mediated restriction of HIV-1. In the absence of viral countermeasures, A3G packages into nascent viral particles and then, during the early stages reverse transcription in susceptible target cells, A3G deaminates viral cDNA cytosines to uracils. These uracil lesions template the insertion of adenine bases during subsequent viral DNA synthesis and result in viral genomic strand guanine-to-adenine (G-to-A) mutations. If unhindered, A3G and related enzymes (A3D, A3F, and A3H) can combine to convert up to 10% of viral cDNA cytosines to uracils in a single round of virus replication, effectively inactivating HIV-1 by hypermutation (lethal mutagenesis) [5,6].

The viral infectivity factor (Vif) of HIV-1 preserves viral genomic integrity by neutralizing A3G and related APOBEC3 enzymes (A3D, A3F, and A3H). Vif heterodimerizes with the transcription co-factor CBF-β, recruits a CULLIN-RING ubiquitin ligase complex, directly binds these APOBEC3 enzymes, and triggers their polyubiquitination and degradation (reviewed by Refs. [1–4]). However, many studies have indicated that A3G-mediated hypermutation and Vif-mediated counteraction are at opposing ends of a wide spectrum of outcomes and that in reality, in infected patients in vivo, is likely to lie somewhere in between. First, the viral genomic strand is depleted of guanines (enriched for adenines) resulting in nucleo-base proportions that differ from human genomic DNA, suggesting continuous evolutionary pressure

from APOBEC3 enzymes. Second, many common drug resistance mutations are G-to-A changes at APOBEC3-preferred sites [7,8]. Particularly noteworthy are deep-sequencing studies showing that pre-existing drug resistance mutations could be attributed to A3G even in the presence of a fully functional Vif counter-defense [9]. Additional deep-sequencing studies compared APOBEC3-preferred sites in cell culture experiments with the viral mutation spectra in patient-derived viral sequences and found evidence for APOBEC3-mediated immune escape [10].

The aforementioned observations support the idea that HIV-1 may be "addicted" to the pro-mutagenic activity of the APOBEC3 enzymes in order to maintain high mutation rates and evade potent adaptive immune responses [11]. Thus, in addition to the obvious goal of inhibiting Vif and promoting lethal mutagenesis (therapy by hypermutation), an alternative therapeutic strategy may be blocking APOBEC3 mutagenesis and thereby starving HIV-1 of necessary genetic variation (therapy by hypomutation) [11]. One approach to APOBEC3 inhibition is in developing peptides or peptidomimetics of regions of Vif that directly bind to A3G and related APOBEC3 enzymes. In support of this idea, HIV-1 Vif expression in *Escherichia coli* was reported to inhibit the activity of the AID, which is an APOBEC3-related DNA deaminase essential for antibody gene diversification [12]. HIV-1 Vif has also been shown to block the single-stranded (ss)DNA deaminase activity of A3G in cells and in viral particles [13–15]. These observations motivated screening of Vif-derived peptides for A3G inhibitory activity [14,16]. Two peptides spanning Vif regions 25–39 and 105–119 showed low micromolar inhibitory activities in biochemical assays and even evidence for A3G antagonism in living cells [14–18].

Given these promising activities, we aimed to use Vif-derived peptides as lead molecules for therapeutic development. However, we did not find significant A3G inhibitory activity for Vif peptide 25–39 in our assays, in contrast to the small molecule A3G inhibitors that have previously identified [19,20]. Moreover, although Vif peptide 105–119 and a shorter derivative spanning Vif residues 107–115 showed reproducible A3G inhibitory activity, this proved to be non-specific as an amino acid scrambled derivative

of Vif peptide 107–115 still showed similar activity suggesting a general non-specific inhibitory mechanism. In light of the A3G-interacting surfaces of Vif, now appreciated to span several discontinuous secondary structures [21–24], we conclude that these Vif-derived peptides are unlikely to be useful for further development as A3G inhibitors.

Results

Vif peptide screen

HIV-1 Vif has been subjected to extensive mutation experiments that have defined regions required for interaction with host cellular proteins including the APOBEC3s (A3F, A3G, A3H), CBF-β, and ubiquitin ligase components (CUL5 and ELOC) (Fig. 1) (reviewed by Refs. [1–4]). A previous screen of Vif peptides identified several with A3G inhibitory activity [14,16]. We started our studies by attempting to reproduce this screen using the same library (HIV-1 lab strain NL4–3 Vif peptide library Cat#6446), a fluorescence-based DNA deaminase activity assay [19], and A3G expressed in the context of a soluble extract from HEK-293 cells (assay schematic in Fig. 2a). The majority of Vif peptides failed to significantly alter the activity of A3G in this assay; however, three elicited strong and reproducible inhibition (Fig. 2b). Vif peptide 6024 (H-VKHHMYISG-KAKGWF-OH), which spans residues 25–39, showed the strongest inhibition (>10-fold), and overlapping Vif peptides 6043 (H-DLADQLIHLYYFDCF-OH) and 6044 (H-QLIHLYYFDCFSESA-OH), which span residues 101–115 and 105–119, respectively, showed weaker inhibition. These data indicate that two, non-overlapping regions of Vif have A3G inhibition activity, consistent with prior studies [14,16,18].

Vif25–39 does not inhibit A3G ssDNA cytosine deaminase activity

Vif peptide 25–39 overlaps with previously defined A3G-interacting regions (Fig. 1) [16,22,25–27]. To further investigate the inhibitory potential of this peptide, it was synthesized on solid phase, purified by reverse-phase HPLC, and characterized by
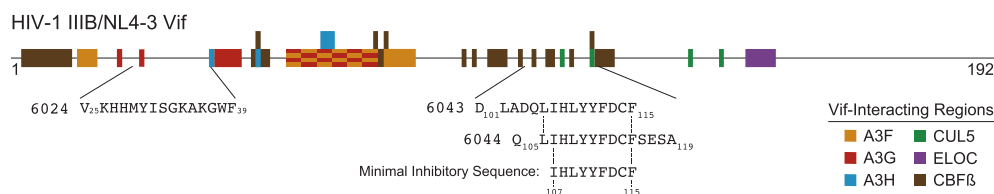

Fig. 1. HIV-1 IIIB Vif schematic depicting relevant interacting regions and peptides. Please see the text for details.
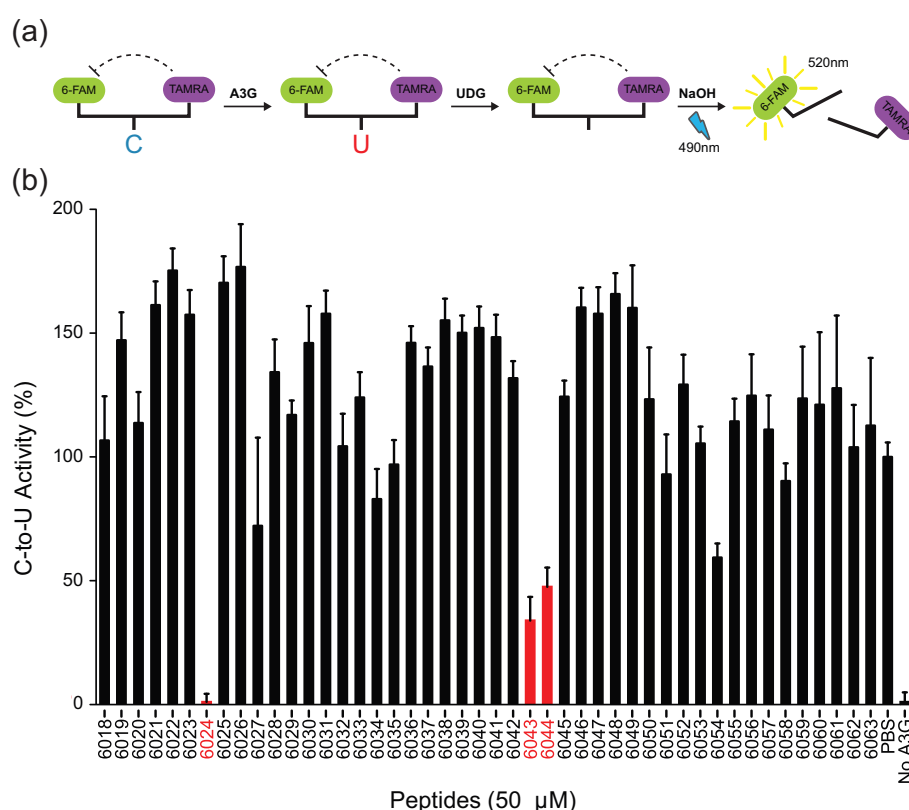
Fig. 2. Effects of Vif-derived peptides on the single-stranded DNA deaminase activity of cellular A3G. (a) Schematic of the fluorescence-based ssDNA deaminase activity assay. 6-FAM fluorescence is quenched by TAMRA in intact substrate ssDNA. The concerted action of ssDNA cytosine deamination by A3G, uracil excision by UDG, and abasic site cleavage by a hydroxide ion releases the 6-FAM from TAMRA quench and yields a strong fluorescence signal. (b) A3G activity in the presence of 50 μM of the indicated 15-mer Vif peptides. Reactions with 293 T-cell extract lacking A3G (no A3G) or containing A3G with solvent only (PBS) are shown as controls. Each histogram bar reports the mean A3G activity ± SEM for two independent experiments.

analytical HPLC for purity (99% pure) and the structure validated by mass spectrometry (Table 1). The resulting peptide was then tested *in vitro* against A3G-myc-His purified from 293 T cells using a fluorescently labeled ssDNA substrate with a single A3G-preferred 5'CC$\underline{C}$ target site. In comparison to

the small molecule MN30 used as a positive control [19], previously published data indicating a 0.6 μM IC$_{50}$ for Vif 25–39 [14], and our own screening data above, the pure Vif peptide 25–39 showed no inhibitory activity and may even stimulate A3G activity at higher concentrations (Fig. 3a).

Table 1. Sequence, purity, and mass spectrometry data for peptides used in this study

Peptide name	Peptide sequence[a]	Mass calc'd (*m/z*)	Mass found (*m/z*)	Purity (215 nm/254 nm)
Vif25–39	H-VKHHMYISGKAKGWF-OH	895.0 (M + 2H)	895.8 (M + 2H)	99%/99%
Vif107–115	H-IHLYYFDCF-OH	1220.5 (M + H)	1220.8 (M + H)	99%/97%
Vif107–115r	H-FCDFYYLHI-OH	1220.5 (M + H)	1220.8 (M + H)	96%/97%
Vif107–115s	H-ICFLHYDFY-OH	1220.5 (M + H)	1220.9 (M + H)	99%/90%
				Purity (220 nm)[b]
6024	H-VKHHMYISGKAKGWF-OH	895.0 (M + 2H)	894.56 (M + 2H)	88.8%
6043	H-DLADQLIHLYYFDCF-OH	1875.9 (M + H)	1875.14 (M + H)	94.2%
6044	H-QLIHLYYFDCFSESA-OH	1835.8 (M + H)	1836.07 (M + H)	82.3%

[a] Sequences shown are written left to right: N-terminus → C-terminus. "H-" designates a free N-terminal amine on the peptide. "-OH" designates the peptide has a C-terminal carboxylic acid.
[b] Purity and mass spectrometry analyses for peptides 6024, 6043, and 6044 were done by Fisher BioServices Inc. as part of contracted quality control by the AIDS Reagent Program.
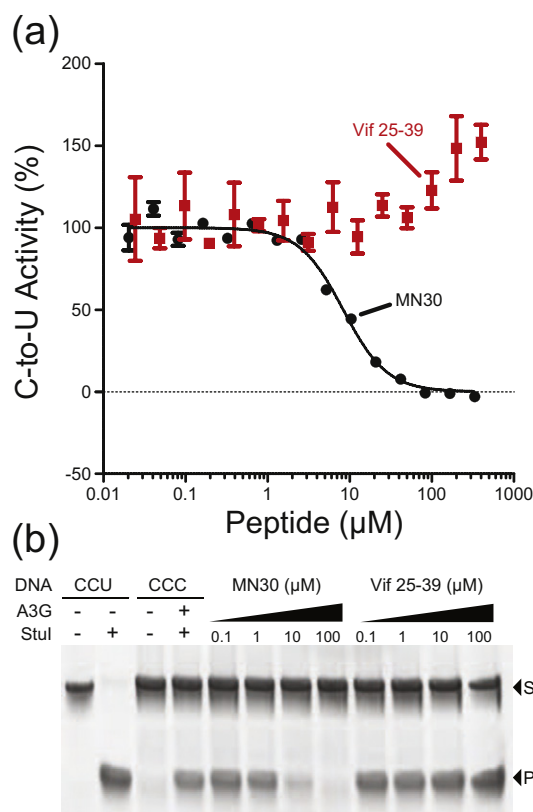
(a)

(b)

Fig. 3. Vif peptide 25–39 does not inhibit A3G ssDNA deaminase activity. (a) Representative dose–response experiment for A3G activity in the presence of the indicated concentration of Vif peptide 25–39. Each data point represents the mean level of A3G activity ± SEM in the fluorescence-based ssDNA deaminase assay (n = 3). Data for the small molecule MN30 are shown as a positive control for inhibition. (b) A3G activity in the presence of the indicated concentration of Vif25–39 using the PCR/restriction-based assay to quantify ssDNA deaminase activity. MN30 is again used as a positive control for inhibition (S, substrate; P, product). This gel image is representative of more than three independent experiments.

To eliminate the possibility of an assay-specific effect, we repeated the experiment using a polymerase chain reaction (PCR) and restriction-based ssDNA deaminase assay used previously [28] and still found no inhibitory effect (Fig. 3b). Finally, the peptide was again synthesized and purified in-house, as well as ordered from a commercial source, and in both cases, again no A3G inhibition was observed. We conclude that Vif peptide 25–39 does not inhibit the ssDNA cytosine deaminase activity of A3G. We note that published results [14] as well our own screening results (Fig. 2b) may be due to an impurity because Vif peptides from the AIDS Reagent Program vary in purity from 82.3% to 94.2% according to specifications documentation (Table 1).

Vif-derived peptide 107–115 non-specifically inhibits A3G ssDNA deaminase activity

We then turned our attention to Vif peptide 107–115, which is defined by the overlapping region between AIDS Reagent Program peptides 6043 and 6044 and previously shown to similarly inhibit A3G ssDNA deaminase activity [18] (Fig. 1). Utilizing the procedure described above, this peptide was synthesized on solid phase and characterized as >97% pure (Table 1). It was then tested against recombinant A3G-myc-His using both the fluorescence-based ssDNA deamination assay [19] and the PCR and restriction-based assay [28] (Fig. 4). With the fluorescence-based assay, Vif peptide 107–115 showed weak inhibitory activity ($IC_{50} > 100$ μM), which was considerably less than the positive control MN30 and a recently reported value for the same peptide ($IC_{50} = 0.1$ μM [18]). With the PCR and restriction-based assay, Vif peptide 107–115 showed no reproducible inhibitory effect. This differential outcome may be due to the fact that the latter assay is semi-quantitative and potentially susceptible to amplification biases from the PCR procedure.

We next asked whether Vif peptide 107–115 inhibited A3G in a sequence dependent manner. This was done by synthesizing scrambled and reverse versions of Vif 107–115 peptide and testing those peptides in both the fluorescence- and PCR/restriction-based DNA cytosine deaminase assays. With both peptides in both assays, similar weak inhibitory effects were observed (Fig. 4a and b). To corroborate these data, the peptides were again synthesized, characterized for purity and correct mass, then retested, ultimately yielding similar results. We conclude that Vif peptide 107–115 is a nonspecific inhibitor of A3G ssDNA deaminase activity, most likely by making general hydrophobic interactions with the enzyme.

Order of addition ssDNA binding studies

We next performed a series of electrophoretic mobility shift assays (EMSAs) to ask whether Vif peptide 107–115 blocks A3G activity by preventing the enzyme from binding to ssDNA substrates. A3G has strong ssDNA binding activity, which is mostly governed by the N-terminal pseudo-catalytic domain with a relatively modest contribution from the C-terminal catalytic domain [19,28–33]. The initial round of experiments was done by first mixing ssDNA with peptide, incubating 15 min, adding enzyme, and then fractionating the complexes on native polyacrylamide gels (EMSA; Fig. 5a). This approach yielded uniformly negative data. However, we next wondered if order of addition makes a difference, and first mixed A3G with peptide, incubated 15 min to optimize opportunities for inhibitor
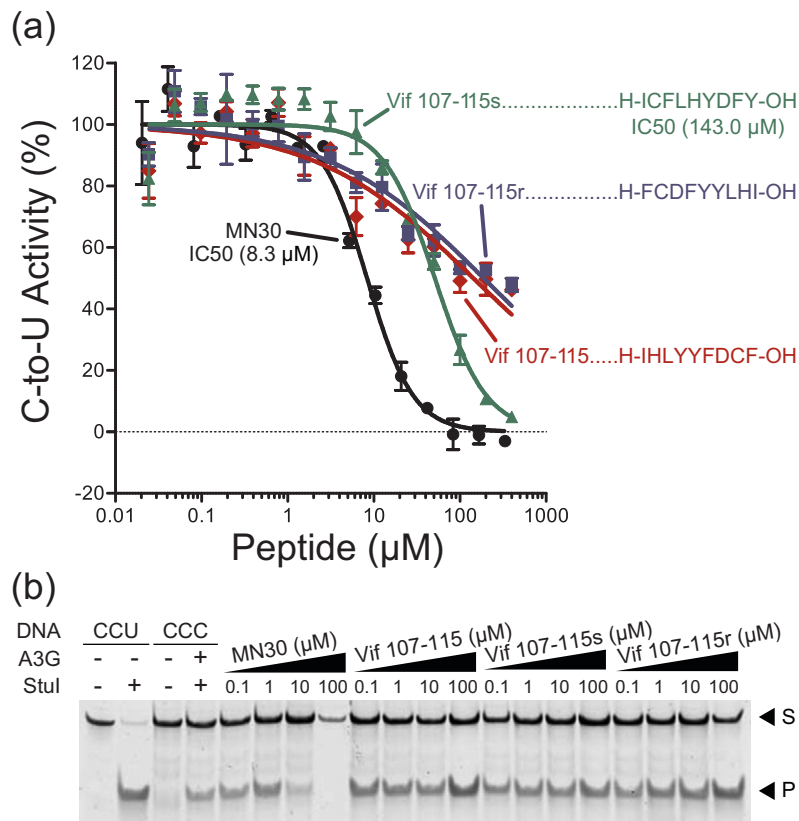
(a)

(b)

Fig. 4. Vif peptide 107–115 inhibits A3G but this activity is non-specific. (a) Dose–response studies of A3G activity in the fluorescence-based assay with the indicated concentrations of Vif-derived peptides or derivatives as described in the main text (red, Vif 107–115; green, Vif 107–115 scrambled; dark blue, Vif 107–115 reverse). Data for the small molecule MN30 are shown as a positive control for inhibition. (b) A3G activity in the presence of the indicated peptides using the PCR/restriction-based assay to quantify ssDNA deaminase activity. MN30 is again used as a positive control for inhibition (S, substrate; P, product). This gel image is representative of more than three independent experiments.

binding, added ssDNA, and then fractionated the complexes on native polyacrylamide gels (Fig. 5b). In this scenario, Vif peptide 107–115 prevented A3G from binding ssDNA in a dose-dependent manner. Given the non-specific inhibition mechanism indicated above, one possible explanation for this phenomenon is that hydrophobic peptides like Vif 107–115, given sufficient time, will coat an enzyme non-specifically and result in steric occlusion of potential ssDNA substrates (Fig. 5c). In contrast, if stable A3G ssDNA complexes are first allowed to form then the peptide will not have a chance to bind and will be too weak to outcompete natural ssDNA substrates (Fig. 5c).

Discussion

In this study, we pursued the idea that the ssDNA cytosine deaminase activity of A3G may be inhibited by Vif-derived peptides. Prior studies reported sub-micromolar inhibitory activities for Vif peptide 25–39 and Vif peptide 105–119, as well as inhibitory activity for derivative Vif peptide 107–115 [14–18]. Although high concentration screening with crude peptide mixtures initially confirmed these results, subsequent experiments with freshly synthesized and rigorously characterized material were either completely negative (Vif 25–39) or weakly positive through a non-specific mechanism (Vif 107–115). Thus, further development of these Vif-derived peptides for therapeutic inhibition of A3G is unlikely to be fruitful.

However, although the present studies are discouraging, they will hopefully caution others from going down similar paths. In addition, our studies may help to advance the overall concept of inhibiting A3G by exploiting the evolutionarily optimized interaction with HIV-1 Vif. Rapid progress in structural studies of the APOBEC3–Vif interaction is already yielding clues for the rational design of non-linear Vif peptides (e.g., artificially joining polypeptide sequences from different structural regions) [21–24]. However, the success of such structure-guided approaches will ultimately require high-resolution knowledge of the different APOBEC3–Vif interfaces and, although individual structures of APOBEC3 enzymes and of the Vif/CBF-β ligase complex now exist, structures of the larger macromolecular complex with both interaction partners have proven elusive.

Finally, it is noteworthy that APOBEC3 inhibition could be both an effective anti-viral strategy and an effective anti-cancer strategy. Recent studies have demonstrated that APOBEC signature mutations are the second largest source of somatic mutation
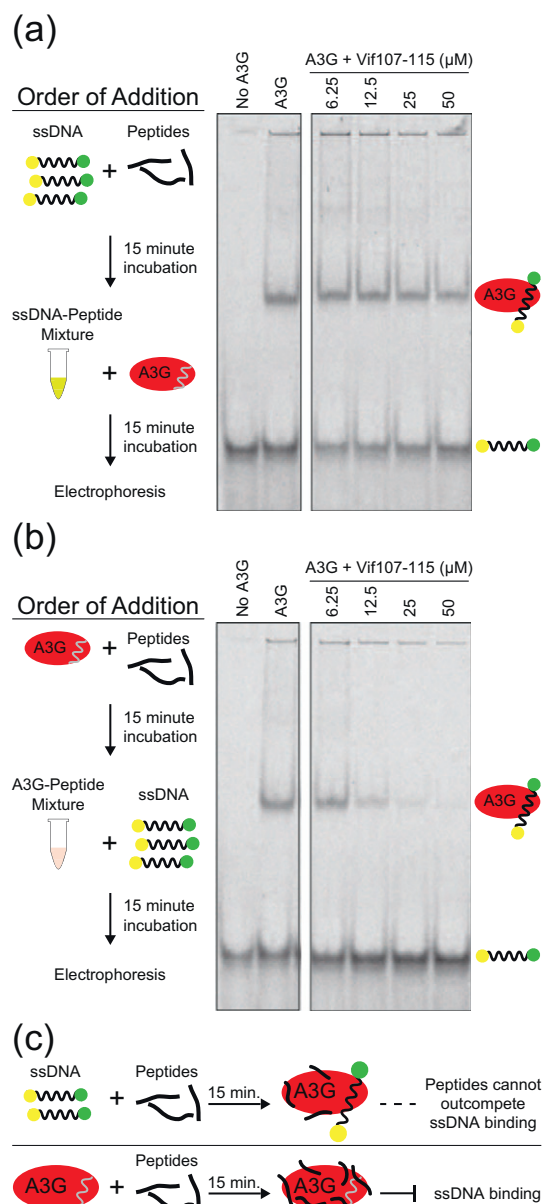
Fig. 5. Reagent addition order influences the ssDNA binding activity of A3G. (a) Representative EMSA showing the ssDNA binding activity of A3G in reactions in which Vif-derived peptides were first incubated 15 min with ssDNA and then A3G was added for an additional 15-min incubation period, followed by native gel fractionation and imaging to detect free ssDNA (lower band) and ssDNA/A3G complexes (upper band). (b) Representative EMSA showing the ssDNA binding activity of A3G in reactions in which Vif-derived peptides were first incubated 15 min with A3G and then ssDNA was added for an additional 15-min incubation period, followed by native gel fractionation and imaging to detect free ssDNA (lower band) and ssDNA/A3G complexes (upper band).

in cancer (second only to the "aging signature" caused by spontaneous, water-mediated deamination of methyl-cytosine nucleobases in CpG motifs)

[34–37]. Moreover, some SIV Vif variants are potent inhibitors of human APOBEC3B [38], which is likely the major enzyme responsible for APOBEC signature mutations in cancer. Thus, the overall strategy suggested here of splicing together non-linear Vif peptide epitopes and developing peptidomimetics may still prove useful in the long-term to block APOBEC3B activity, slow rates of tumor evolution, and improve the durability of existing cancer therapies.

Materials and Methods

A3G expression and purification

A3G-mycHis was purified from stably or transiently transfected HEK293T cells using the C-terminal hexahistidine tags, as described [19,39]. Cells were maintained in DMEM (Invitrogen) with 10% FBS (Hyclone), 50 units/ml penicillin and 50 μg/ml streptomycin (Invitrogen) in 37 °C and 5% CO_2. Transfections were done with TransIT-LTI (Mirus Bio). Forty-eight hours post-transfection, cells were harvested and lysed in 25 mM Hepes (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, 1 mM EDTA, 0.5% Triton X-100, and 10% glycerol. Insoluble materials were removed from crude cell lysates by centrifugation (14,000 rpm, 10 min) and A3G was purified from supernatants.

DNA deaminase activity assays and peptide screens

The fluorescence-based DNA cytosine deamination assay was used to monitor A3G activity in 293 T-cell lysates, as described [19], and was adapted as follows for work with purified proteins. Recombinant human A3G-mycHis was diluted with 50 mM Tris–Cl (pH 7.4), 150 mM NaCl, 10% glycerol, 0.5% triton X-100, 1 mM PMSF (MP Biomedicals), 1 μg/ml Aprotinin (Sigma), 1 μg/ml Leupeptin (MP Biomedicals), and 1 μg/ml Pepstatin A (Fisher Scientific) to working concentrations. Fifteen microliters of enzyme was incubated with 10 pmol ssDNA substrate 5′-6-FAM-AAA-CCC-AAA-GAG-AGA-ATG-TGA-TAMRA-3′ (Biosearch Technologies, Inc.) for the initial peptide screen and 5′-6-FAM-AAA-TAT-CCC-AAA-GAG-AGA-TAMRA-3′ (Biosearch Technologies, Inc.) was used for follow-up dose–response experiments. In both reaction series, ssDNA deamination products were then treated with 0.02 units of uracil DNA glycosylase (UDG; NEB) diluted in 15 μl of 50 mM Tris–Cl (pH 7.4) and 10 mM EDTA for 2 h at 37 °C in Nunc 384-well black plates. Three microliters of 4 N NaOH was added, followed by mixing and incubating at 37 °C for another 30 min. Three microliters of 4 N HCl

and 37 µl of 2 M Tris–Cl (pH 7.9) was then added for neutralization, and the relative deaminase activity was quantified by reading fluorescence with excitation at 490 nm and emission at 520 nm on Synergy Mx Monochromator-Based Multi-Mode (BioTek Instruments, Inc.) or LJL Analyst AD (LJL BioSystems, Inc.) microplate readers. UDG assays omitted the deaminase and used ssDNA substrate with a single uracil in place of the A3G-preferred cytosine (5′-6-FAM-AAA-CCUAAA-GAG-AGA-ATG-TGA-TAMRA-3′). All compound stocks were suspended in 10 mM DMSO and diluted as indicated. High-throughput screening used 0.04 µM A3G, 0.33 µM ssDNA substrate, 10 µM compound, and 0.02 units UDG (NEB). IC$_{50}$ values were determined using GraphPad Prism 6.0 software.

EMSAs

A3G-mycHis was incubated with ssDNA substrate 5′-biotin-ATT-ATT-ATT-ATT-CCA-ATG-GAT-TTA-TTT-ATT-TATTTA-TTT-ATT-T-fluoroescein-3′ in 50 mM Tris–Cl (pH 7.8), 100 mM KCl, 10 mM MgCl$_2$, 1 mM DTT, 0.5 mM ZnCl$_2$, and 10% glycerol at 37 °C for 30 min with varying concentrations of compounds in DMSO. The reaction products were subjected to native TBE PAGE. A3G ssDNA complexes were visualized at 463 nm using a Fuji FLA-5000 fluorescent image analyzer.

PCR-based ssDNA deamination assay

A3G deamination reactions were performed as described [28]. A3G-catalyzed DNA deamination of 5′-AGGCCC-3′ results in 5′-AGGCCU-3′, which after PCR amplification is converted to 5′-AGGCCT-3′ which is the recognition site for Eco147I/StuI restriction digestion. A3G (10 nM) was incubated with 1 nM ssDNA (5′-GGATTGGTTGGTTATTTGT TTAAGGAAGGTGGATTAA**AGGCCC**AATAAGGT GATGGAAGTTATGTTTGGTAGATTGATGG-3′; Integrated DNA Technologies) in a total volume of 10 µl in 25 mM Tris–Cl (pH 7.0) and 0.1 mg/ml bovine serum albumin at 37 °C for 30 min. The reaction was terminated by heating to 95 °C for 5 min. One microliter of the reaction mixture was used for PCR amplification in a 20-µl reaction volume with 1× PCR buffer containing 1.5 mM MgCl$_2$, 1 µM forward 5′-GGATTGGTTGGTTATTTGTTTAA GGA-3′ and reverse 5′-CCATCAATCT ACCAAACA TAACTTCCA-3′ primers, 0.2 mM dNTP, and 1 unit of Choice Taq DNA polymerase (Denville Scientific Inc.). The PCR program was 1 cycle at 95 °C for 3 min followed by 15 cycles of annealing at 61 °C for 30 s and denaturing at 94 °C for 30 s. Ten microliters of each PCR product was incubated with Eco147I/StuI restriction enzyme (NEB) for 1 h at 37 °C. Oligo containing CCU instead of CCC was used as positive control for deamination. Restriction-reaction products were loaded onto 14% polyacryl-amide gels and separated by electrophoresis in 1× TBE. Gels were stained with SYBR gold nucleic acid stain (Invitrogen) diluted 1:10,000 in 0.5× TBE and imaged in Typhoon FLA7000 laser scanner (GE).

Solid-phase peptide synthesis

Automated solid-phase peptide synthesis was performed using a Protein Technologies PS3 peptide synthesizer as reported [40]. Pre-loaded resins (H-Phe-2-ClTrt-Resin [200–400 mesh] 0.69 meq g^{-1}; H-Ile-2-ClTrt-Resin [100–200 mesh] 0.67 meq g^{-1}; H-Tyr(tBu)-2-ClTrt-Resin [100–200 mesh] 0.67 meq g^{-1}) purchased from Peptides International were used for solid-phase peptide synthesis. Protected resin-bound peptides were cleaved from resin using a solution (10 ml) of trifluoroacetic acid/distilled water/triisopropyl silane/ethane dithiol (85:5:5:5) and precipitated with cold diethyl ether (30 ml). The white precipitate was isolated by centrifugation (4000 rpm, 5 min, 25 °C). The crude peptide was purified *via* semi-preparative HPLC chromatography using distilled and deionized H$_2$O (with 0.1% TFA) and an increasing gradient of MeCN (with 0.1% TFA) on a Zorbax SB-C18 column (21.2 × 250 mm, 7 µm; Agilent Technologies). After purification, the peptide was analyzed by analytical HPLC for purity (215 and 254 nm) and characterized by electro-spray ionization mass spectroscopy in positive ion mode on an Agilent 1100 Series LC/MSD Ion Trap instrument. See Table 1 for the purities of individual peptides and their calculated and observed masses.

Synthetic oligonucleotides and purchased peptides

The sequence of the 80-mer ss-deoxyoligonucleotide substrate used in the deamination assays is as follows:
5′-GGATTGGTTGGTTATTTGTTTAAGGAA GGTGGATTAAAGGCCCAATAAGGTGATG GAAGTTATGTTTGGTAGATTGATGG-3′ (the A3G-preferred target cytosine is underlined). The positive control ssDNA has the same sequence except the target C is replaced by a U. The following primers were used for PCR amplification of the substrate and positive control nucleotides:
Forward 5′-GGATTGGTTGGTTATTTGTTTAA GGA-3′;
Reverse 5′-CCATCAATCTACCAAACATAACT TCCA-3′
Vif-derived peptides were obtained through the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program as lyophilized powder and were subsequently dissolved in water or a DMSO solution (Vif peptide set Cat#6446[†]). Peptides were also purchased from GL Biochem

(Shanghai, China) (data from these peptides are not shown, but available upon request).

HIV-1 IIIB/NL4–3 Vif sequence (GenBank EU541617)

MENRWQVMIVWQVDRMRIRTWKSLVKHHMYI
SGKAKGWFYRHHYESTHPRISSEVHIPLGDARLV
ITTYWGLHTGERDWHLGQGVSIEWRKKRYSTQV
DPDLADQLIHLYYFDCFSESAIRNAILGHIVSPRCE
YQAGHNKVGSLQYLALAALITPKKIKPPLPSVTKL
TEDRWNKPQKTKGHRGSHTMNG.

Conflict of Interest

D.A.H. and R.S.H. are co-founders, shareholders, and consultants of ApoGen Biotechnologies, Inc.

Present address (A.L.P.): Department of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, MN 55455, USA.
Present address (A.R.): The Aaron Diamond AIDS Research Center, 455 First Avenue, New York, NY 10016, USA.

†http://www.aidsreagent.org/support_docs/6446_lot_140319_and_12539_lot_140222_Con_B_Vif_Analytical_Data.pdf

Abbreviations used:
A3G, APOBEC3G; AID, activation-induced deaminase; CBF-β, core-binding factor beta; EMSA, electrophoretic mobility shift assays; G-to-A, guanine-to-adenine; HPLC, high-performance liquid chromatography; IC_{50}, half maximal inhibitory concentration; MS, mass spectrometry; PCR, polymerase chain reaction; ssDNA, single-stranded DNA; UDG, uracil DNA glycosylase; Vif, viral infectivity factor.

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