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Brief Communication

Estimating the fraction of progeny virions that must incorporate APOBEC3G for suppression of productive HIV-1 infection

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ABSTRACT

The contest between the host factor APOBEC3G (A3G) and the HIV-1 protein Vif presents an attractive target of intervention. The extent to which the A3G–Vif interaction must be suppressed to tilt the balance in favor of A3G remains unknown. We employed stochastic simulations and mathematical modeling of the within-host dynamics and evolution of HIV-1 to estimate the fraction of progeny virions that must incorporate A3G to render productive infection unsustainable. Using three different approaches, we found consistently that a transition from sustained infection to suppression of productive infection occurred when the latter fraction exceeded ~0.8. The transition was triggered by A3G-induced hypermutations that led to premature stop codons compromising viral production and was consistent with driving the basic reproductive number, R_0, below unity. The fraction identified may serve as a quantitative guideline for strategies targeting the A3G–Vif axis.

Introduction

The host protein APOBEC3G (A3G), a cytidine deaminase, induces significant GG-to-AG hypermutations in HIV-1, which in the absence of the HIV-1 protein Vif dramatically inhibit infection in vitro (Malim, 2009). Vif targets A3G for proteasomal degradation, suppressing hypermutations and allowing persistent infection. The A3G–Vif interaction is thus a remarkable example of the evolutionary arms race between retroviruses and their hosts (Compton et al., 2012) and a promising target of intervention (Dapp et al., 2012; Harris and Liddament, 2004). Indeed, several candidate drug molecules targeting the A3G–Vif axis are currently under development (Cen et al., 2010; Dapp et al., 2012; Ejima et al., 2011; Nathans et al., 2008). The extent to which the A3G–Vif interaction must be blocked in order to tilt the balance in favor of A3G, however, remains unknown.

A3G-induced hypermutations may result in premature stop codons that potentially suppress the production of infectious progeny virions. For instance, the codon for tryptophan (TGG) can be converted to a stop codon (TAG). Although proviral DNA sequences often show high levels of hypermutation (Keele et al., 2008; Kieffer et al., 2005; Land et al., 2008; Piantadosi et al., 2009), the proportion of viral RNA sequences carrying extensive hypermutations in peripheral blood is small (Kieffer et al., 2005; Land et al., 2008), suggesting that the production of virions containing genomes with hypermutations/stop codons may be suppressed (Kieffer et al., 2005; Russell et al., 2009). In agreement, in vitro studies detected progressively fewer genomes with hypermutations in going from proviral DNA to cellular RNA and viral RNA, indicating a strong bias against packaging genomes with extensive hypermutations into progeny virions (Russell et al., 2009). As the fraction of virions carrying A3G rises, the resulting reduction in the pool of infectious virions may render productive infection unsustainable.

A3G molecules expressed in HIV-1-infected cells are incorporated into budding virions. In the absence of Vif, between 4 and 26 A3G units are estimated to be packaged per virion (Armitage et al., 2012; Browne et al., 2009; Nowarski et al., 2008; Xu et al., 2007). Vif appears to restrict A3G incorporation to 0.3–0.8 units/virion (Nowarski et al., 2008), potentially ensuring that an adequate number of virions that do not carry A3G is produced, which can productively infect cells and sustain infection. Candidate drug molecules targeting the A3G–Vif axis either suppress Vif, upregulate A3G expression or activity, or block the interaction of Vif and A3G (Cen et al., 2010; Dapp et al., 2012; Ejima et al., 2011; Nathans et al., 2008), in each case resulting effectively in an increased fraction of budding virions incorporating A3G. Our goal therefore was to identify the minimum fraction of progeny virions that must incorporate A3G in order to render productive HIV-1 infection

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unsustainable. We employed stochastic simulations and mathematical modeling to identify this critical fraction.

Results

Our simulations mimicked the within-host dynamics of productive HIV-1 infection using a population genetics framework. The simulations have been shown previously to capture HIV-1 diversification in patients quantitatively (Balagam et al., 2011; Vijay et al., 2008). Here, we modified the simulations to account for the influence of intervention at the A3G–Vif axis. Briefly, we created an initial pool of virions, each carrying 2 copies of the full-length HIV-1 genome (9073 nucleotides; GenBank: JN024117.1). A fraction a of the virions was assumed to carry A3G. We selected virions randomly from this pool and synchronously infected a pool of uninfected cells. The viral RNA was then reverse transcribed into proviral DNA, during which process mutation and recombination occurred. When an infecting virion contained A3G, hypermutations were also introduced at a rate dependent on the position on the genome, following the twin-gradient hypermutation pattern observed experimentally (Kijak et al., 2008; Suspene et al., 2006; Yu et al., 2004). The resulting mutated, recombined, and hypermutated proviral DNA in each cell were then transcribed into viral RNA, which were assorted into pairs and released as progeny virions. A fraction a of the progeny virions was again assumed to carry A3G. The progeny virions formed the new viral pool from which virions were chosen according to their relative fitness for the infection of a fresh set of uninfected cells, and the cycle was repeated (Fig. 1). We tracked the evolution of the sizes of infected cell and viral pools and the accumulation of mutations with time (or generations) and averaged these quantities over many realizations. Supplementary material S1 contains details of the simulation procedure and the parameter values employed. We examined the implications of increasing a on the sustainability of infection.

Interestingly, we found a sharp transition from sustained infection to complete suppression of infection as a was increased. With $a < 0.8$ the size of the infected cell pool remained nearly constant indicating sustained infection (Fig. 2A). As a increased to 0.8 and beyond, the population decreased and eventually vanished, marking the extinction of infection. We identified the critical fraction of virions that must incorporate A3G as $a_c \approx 0.81$ as that value of a beyond which infection went extinct in $>90\%$ of the realizations in our simulations. The time for infection to be driven extinct reduced exponentially as a increased beyond a_c (Fig. 2B).

We validated this estimate of a_c in 2 independent ways, namely, using a population genetics-based mathematical model and using the basic model of viral dynamics. Using simulations we deduced that the impact of A3G on the sustainability of infection was determined primarily by the induction of premature stop codons; other factors, such as mutation, recombination, and fitness penalties associated with hypermutations that did not result in stop codons, had only a minor influence (Supplementary material S2; Fig. S1). Accordingly, we constructed a population genetics-based mathematical model that explicitly accounted for the impact of A3G-induced stop codons on the population of productively infected cells and hence on the sustainability of infection (Supplementary material S3). The model yielded

$$a_c = \frac{1}{1-\prod_{i=1}^{s}(1-\mu_h[l_i])}\left(1-\frac{1}{2\left(1-\left\langle\frac{1}{M}\right\rangle\right)}\left(-\left\langle\frac{1}{M}\right\rangle+\sqrt{\left\langle\frac{1}{M}\right\rangle^2+4\left(1-\left\langle\frac{1}{M}\right\rangle\right)\frac{\langle M\rangle}{\langle f\rangle P}}\right)\right) \quad (1)$$

indicating that a_c depended on the position-dependent hypermutation rate, $\mu_h[l_i]$, where l_i is the position of the *ith* of the s potential stop codon sites on the viral genome; the arithmetic and harmonic means, $\langle M\rangle$ and $\langle 1/M\rangle$, respectively, of the number of infections/cell; the number of progeny virions/cell, P; and the mean relative fitness of the progeny viral pool, $\langle f\rangle$. Scanning the full-length HIV-1 genome employed in our simulations for loci where GG-to-AG hypermutations would lead to stop codons (e.g., TGG sites), we found that the genome contained $s \sim 10^2$ such potential stop codon sites, which with the position-dependent hypermutation rate, $\mu_h[l]$, implied $\prod_{i=1}^{s}(1-\mu_h[l_i]) < 10^{-3}$. (We examined three other HIV-1 genomes, viz., GenBank: JN397362.1, JQ403032.1 and JF320530.1, and found a similar number of potential stop codon sites). We obtained $\langle M\rangle$ and $\langle 1/M\rangle$ from the distribution of the frequency of multiple infections employed in our simulations (Supplementary material S1), which mimics recent experimental observations suggesting that a majority of infected cells is singly infected (Josefsson et al., 2011; Schultz et al., 2012).

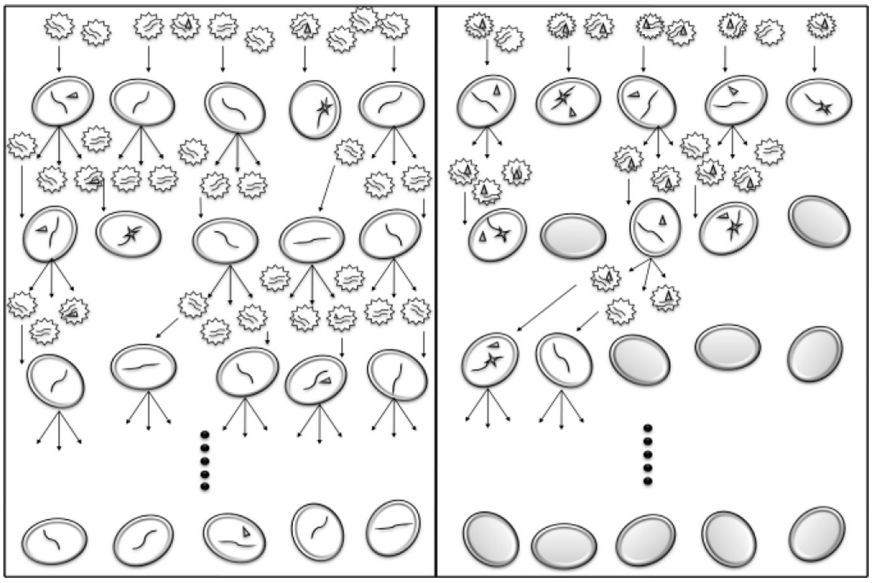

Fig. 1. Schematic of the progression of infection and the influence of A3G. Viral particles with a fixed fraction containing A3G (triangles) infect cells. The resulting proviral DNA can contain stop codons (crosses) if the infecting virion contains A3G. Proviral DNA containing stop codons do not yield infectious progeny virions. When the fraction of virions containing A3G is small, infection is sustained (left), whereas when it crosses a critical value, infection dies out (right).

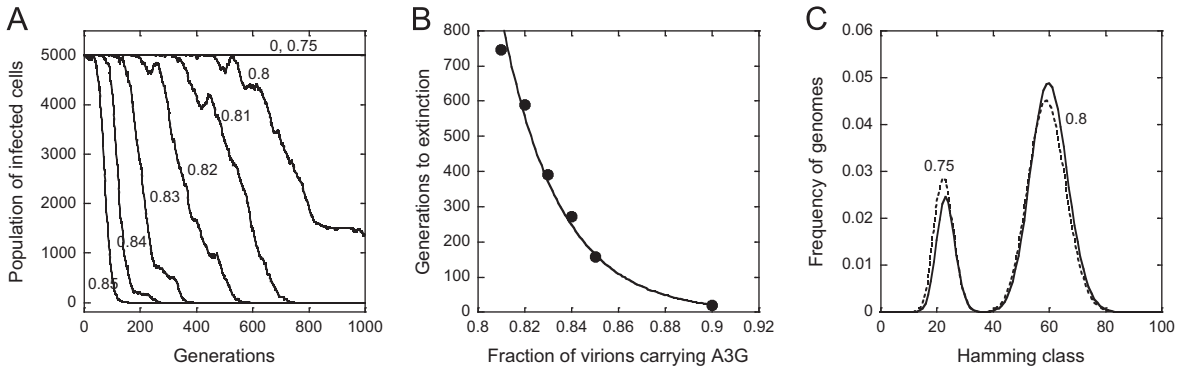

Fig. 2. A3G-induced suppression of productive infection. (A) Time-evolution of the population of infected cells predicted by our simulations for different values of a, the fraction of progeny virions containing A3G, indicated. (B) The corresponding number of generations at which infection became extinct from the start of intervention (symbols) fit to an exponential profile (line). (C) The structure of the quasispecies determined from (A) for $a=0.75$ (dotted line) and $a=0.8$ (solid line). For $a=0.8$, the realizations in which the infection was sustained were employed. Parameter values employed are mentioned in Supplementary material S1.

To estimate $\langle f \rangle$, we employed the structure of the quasispecies predicted by our simulations as a approached a_c. The quasispecies displayed a bimodal structure, with peaks at Hamming classes $\sim$20 and $\sim$60 (Fig. 2C). The 2 peaks represented the 2 kinds of viral particles in the viral pool, one carrying A3G and the other not. Genomes from the former virions suffered hypermutations upon infecting cells, whereas genomes from the latter did not. Accordingly, the quasispecies structure, based on the resulting proviral DNA, displayed 2 peaks. Most genomes comprising the second peak are expected to contain premature stop codons (given $s \sim 10^2$) and are thus not likely to be packaged in progeny virions. We therefore estimated $\langle f \rangle$ as the mean fitness of the portion of the quasispecies under the first peak. We found that $\langle f \rangle \approx 0.76$, using which in Eq. (1) along with other parameters employed in our simulations (Supplementary material S1) yielded $a_c \approx 0.815$, in close agreement with the value obtained from our simulations.

Next, we advanced the basic model of HIV dynamics to account explicitly for A3G activity. The basic model has been employed successfully to describe viral load changes in patients, derive insights into disease pathogenesis, and assess treatment efficacy (Nowak and May, 2000; Perelson, 2002). In particular, it predicts that persistent infection requires that the basic reproductive number, R_0, defined as the number of cells infected by virions produced from one infected cell in a wholly susceptible cell population, be larger than unity (Nowak and May, 2000). Here, we estimated a_c as the minimum value of a at which R_0^t, the value of R_0 under treatment targeting the A3G–Vif axis, dropped below unity (Supplementary material S4). We obtained

$$a_c = \frac{1}{1-\prod_{i=1}^{s}(1-\mu_h[l_i])}\left(1-\frac{1}{R_0}\right), \qquad (2)$$

which because $\prod_{i=1}^{s}(1-\mu_h[l_i]) < 10^{-3}$ simplified to $a_c \approx 1-1/R_0$. Using recent estimates of $R_0 \sim 6$ (Stafford et al., 2000) and ~ 8 (Ribeiro et al., 2010), derived from patient data during acute infection, we obtained $a_c \approx 0.83-0.875$, again similar to our estimate above.

Thus, using three approaches, namely, stochastic simulations of viral evolution, population genetics-based mathematical modeling and viral dynamics-based mathematical modeling, we estimated that when over approximately 80% of the progeny virions incorporated A3G, productive HIV-1 infection was rendered unsustainable.

Discussion

Lethal mutagenesis holds promise as a novel strategy for the treatment of HIV-1 infection (Dapp et al., 2012; Lauring and Andino, 2010; Mullins et al., 2011). Recognizing that A3G is a natural mutagen of HIV-1 that is suppressed by Vif, the A3G–Vif interaction is being targeted with the hope of unleashing the mutagenic potential of A3G. A recent multi-scale model (Hosseini and Mac Gabhann, 2012) has examined the relative merits of suppressing Vif, upregulating A3G expression or activity, or blocking the interaction of Vif and A3G, representing different modes of targeting the A3G–Vif axis employed by current drug candidates. We recognized here that each of these modes would result effectively in an increased fraction of budding virions incorporating A3G. Accordingly, we estimated the critical fraction of progeny virions that must contain A3G in order to render productive HIV-1 infection unsustainable. We found the fraction to be approximately 0.8, yielding a quantitative guideline for strategies targeting the A3G–Vif axis.

Intriguingly, the critical fraction identified lies at the upper end of the range 0.3–0.8 A3G units/virion observed experimentally with fully functional Vif (Nowarski et al., 2008). Controlled hypermutations may serve to increase viral diversity and potentially accelerate viral adaptation (Berkhout and de Ronde, 2004; Kim et al., 2010; Sadler et al., 2010; Wood et al., 2009). Indeed, suppressing A3G has been suggested as a potential intervention strategy that may restrict viral diversity and prevent immune escape (Harris, 2008). If A3G incorporation increased beyond the critical fraction, however, infection would be rendered unsustainable. Thus, for HIV-1 to derive the maximum benefit from A3G, it would have to maintain the level of A3G incorporation just below the critical fraction, which is what Vif appears to accomplish. The observed 0.3–0.8 A3G units/virion may thus be indicative of an evolutionary optimum attained by HIV-1. We recognize that our simulations yield the critical fraction of virions that must be prevented by A3G from productively infecting cells and not the number of A3G units that must be incorporated within a virion to render it incapable of productive infection. In recent studies, the presence of a single A3G catalytic unit per virion has been found adequate to exert maximal antiviral activity, suggesting an "all-or-nothing" mode of A3G action (Armitage et al., 2012; Browne et al., 2009). The latter scenario remains to be fully established; sublethal A3G activity has also been reported (Sadler et al., 2010). Given that Vif drives the average number of A3G units down from 4–26 to 0.3–0.8 per virion, we may assume that with fully functional Vif, a virion may typically carry at most 1 A3G unit. Then, with the all-or-nothing scenario, the critical fraction in our simulations would be equivalent to the corresponding average number of A3G units per progeny virion. Whether the in vitro estimate of 0.3–0.8 A3G units/virion (Nowarski et al., 2008), obtained by inferences from measurements of deaminase activity rather than direct protein quantification, also holds in vivo remains to be established.

Lethal mutagenesis has to be employed with caution because suboptimal increase in the mutation rate may increase viral diversity and facilitate drug resistance and immune escape. This cautionary note has been sounded for intervention at the A3G–Vif axis as well (Harris, 2008; Malim, 2009; Pillai et al., 2008; Smith, 2011). Indeed, A3G-induced development of drug resistance has been observed in vitro (Kim et al., 2010; Mulder et al., 2008). We speculate based on our simulations that targeting the A3G–Vif axis may be much less fraught with the risk of enhancing viral adaptability than strategies attempting to drive HIV-1 past its error threshold. Our simulations suggest that the viral quasispecies would remain compact upon increasing the fraction of virions carrying A3G to the critical value; infection would be driven to extinction without the significant increase in diversity expected upon crossing the error threshold. For instance, whereas the quasispecies remained localized around Hamming classes $\sim$20 and $\sim$60 in our simulations (Fig. 2C), crossing the error threshold would result in complete delocalization of the quasispecies yielding a mean Hamming class of $L/2 \approx 5000$ (Tripathi et al., 2012). In support, another modeling study has also estimated that A3G would contribute marginally to the increase in mutations at known drug resistance sites (Jern et al., 2009).

We recognize several limitations of our study. First, our simulations did not consider viral reservoirs, such as latently infected cells (Finzi et al., 1999), where the virus can reside long-term without active replication. Our estimate of the critical fraction is thus for suppression of productive infection. Second, we did not consider hypermutations in the GA dinucleotide context as well as other non-editing modes of A3G action against HIV-1 (e.g., see Martin et al., 2011; Norman et al., 2011). Third, we restricted our simulations to A3G, whereas other APOBEC family members, particularly A3F, may also contribute to lethal viral DNA editing and further compromise viral infectivity. Fourth, we assumed that the initial viral pool comprised the fittest genome alone, whereas the viral quasispecies in the chronic phase of infection, when intervention is likely to commence, is expected to comprise a diverse pool of genomes with a lower mean fitness, and hence requiring lower A3G activity than estimated for suppression of infection. Our estimate of the critical fraction is thus conservative and may be treated as an upper bound on the extinction threshold.

In conclusion, our study estimated the minimum fraction of virions that must incorporate A3G to suppress productive infection of HIV-1, presenting a quantitative guideline for strategies targeting the A3G–Vif axis.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2013.11.026.

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