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Brief Communication

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

Pulari U. Thangavelu, Vipul Gupta, Narendra M. Dixit *

Indian Institute of Science, Bangalore 560012, India

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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

* Correspondence to: Department of Chemical Engineering, Indian Institute of Science, Bangalore 560012, Karnataka, India. Tel.: +91 80 2293 2768, +91 98808 42145; fax: +91 80 2360 8121.
E-mail addresses: narendra@chemeng.iisc.ernet.in, nmdixit@gmail.com (N.M. Dixit).

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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).

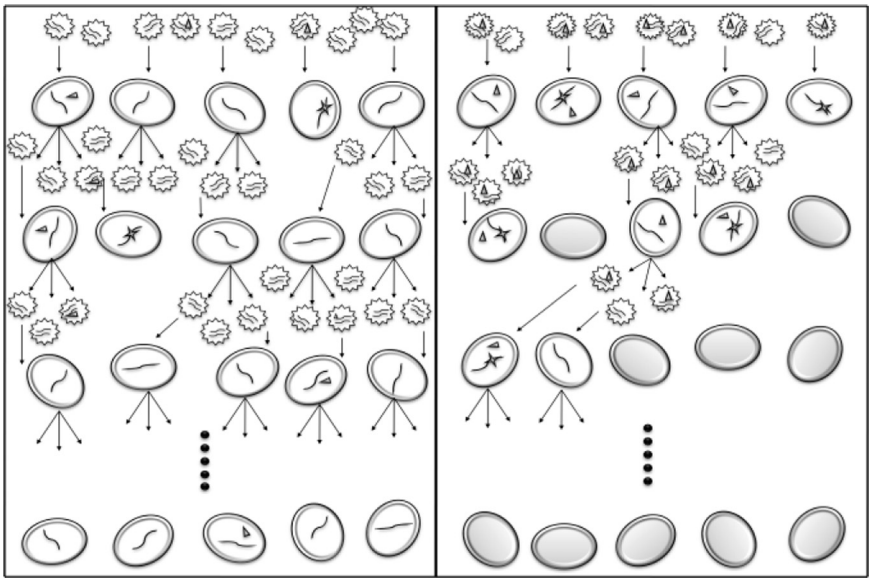

Fig. 1. Schematic of the progression of infection and the influence of A3G. Viral particles with a fixed fraction containing A3G (triangles) infect cells. The resulting proviral DNA can contain stop codons (crosses) if the infecting virion contains A3G. Proviral DNA containing stop codons do not yield 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).