Variation in HIV-1 set-point viral load: Epidemiological analysis and an evolutionary hypothesis

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The natural course of HIV-1 infection is characterized by a high degree of heterogeneity in viral load, not just within patients over time, but also between patients, especially during the asymptomatic stage of infection. Asymptomatic, or set-point, viral load has been shown to correlate with both decreased time to AIDS and increased infectiousness. The aim of this study is to characterize the epidemiological impact of heterogeneity in set-point viral load. By analyzing two cohorts of untreated patients, we quantify the relationships between both viral load and infectiousness and the duration of the asymptomatic infectious period. We find that, because both the duration of infection and infectiousness determine the opportunities for the virus to be transmitted, this suggests a trade-off between these contributions to the overall transmission potential. Some public health implications of variation in set-point viral load are discussed. We observe that set-point viral loads are clustered around those that maximize the transmission potential, and this leads us to hypothesize that HIV-1 could have evolved to optimize its transmissibility, a form of adaptation to the human host population. We discuss how this evolutionary hypothesis can be tested, review the evidence available to date, and highlight directions for future research.

cohort studies | life-history | mathematical model | trade-off | virulence

Viral load, a measure of the density of virus particles in peripheral blood, is an imperfect but important measure of the severity of HIV-1 infection. Although its relationship to virus density in other body compartments and to viral replicative capacity is unclear (1–3), it has a proven track record in the prognosis of patients (4, 5) and has more recently been shown to predict the probability of transmission between discordant couples (6, 7). Viral load is heterogeneous both within patients over time and between patients. During the long asymptomatic period of infection, viral loads fluctuate around a steady set-point value, which varies up to 1,000-fold between patients (4, 5).

Although much work has considered the significance of primary infection in HIV-1 transmission (e.g., refs. 8 and 9), it is not known which set-point viral loads have the greatest epidemiological impact, in terms of leading to the greatest number of infections over the lifetime of the host. Such information is important to determine the public health consequences of targeting prevention efforts at patients with certain subsets of viral loads: high, low, or intermediate. Although it is clear that patients with higher viral loads will be more infectious (6, 7), it is also well known that, untreated, these individuals have a poorer prognosis and, hence, will have fewer lifetime opportunities for transmission (4, 5). The epidemiological impact of different viral loads will hence be determined by the interplay between these two antagonistic processes.

The aim of this study is to use available data on infectiousness (6, 7, 9) and duration of infection (5, 10) to determine, in general terms, the epidemiological impact of cross-sectional variation in viral load. We estimate the product of infectiousness and duration of infection, which we term the transmission potential. This is the mean number of persons one index case can potentially infect over their whole asymptomatic period, estimated as a function of set-point viral load.

We quantify the transmission potential as a function of set-point viral load and find that it is maximized for intermediate viral loads, which we observe are also the most common among untreated patients. Although individuals with high viral loads are the most infectious in the short term, the total contribution to infection of those with intermediate viral loads is found to be larger because of the longer duration of asymptomatic infection. The consequences for public health and an evolutionary hypothesis arising from this observation are discussed.

Results

Set-Point Viral Load and the Duration of Asymptomatic Infection. HIV-1 viral load follows a characteristic U-shape during the course of untreated infection, highest at the start (primary stage) and end (late stage) of infection, whereas lower and relatively steady levels are maintained for a variable number of years during asymptomatic infection. Levels measured in peripheral blood during the asymptomatic period are very variable, ranging from 1,000 to 1 million viral copies per milliliter, and this quantity is positively correlated with viral levels in other body compartments (1–5). We aim to study its relation to the duration of infection using a flexible parametric model in a robust inference framework.

To provide good quantitative detail, we focus on the Amsterdam seroconverters cohort, where homosexual men were recruited prospectively to study the incidence and natural history of HIV-1 infection from 1982 onwards and followed for many years; the cohort has been described elsewhere (5). We censor all observations after 22 November 1993, the date when the first protease inhibitor was used in this cohort, to avoid biases caused by the availability of effective treatment and ignore all treatment effects before this date; our sample size is 123 men, followed for 504 person-years. We determine set-point viral loads as the geometric mean viral load in the interval between the end of primary infection (defined as 6 months after first seropositive sample) and the first AIDS-defining event (CDC type C) or censoring, whichever occurs first. The distribution of these set-points is plotted in Fig. 1, as well as the distribution of viral loads collected from a Zambian cohort (7), referred to in more detail below.

We describe the duration of the asymptomatic stage of

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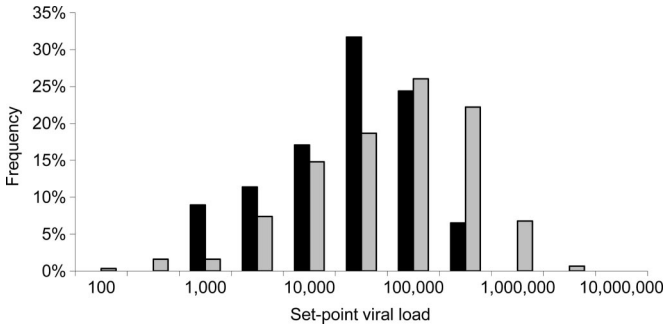

Fig. 1. The distribution of set-point viral loads. The distribution of viral loads (copies per milliliter of peripheral blood) is plotted for untreated individuals in the Amsterdam Seroconverters Cohort (black bars) and the Zambian Transmission Study (7) (gray bars). The bars represent bins 0.5 $\log_{10}$ wide and are labeled by their midpoint viral load.

infection by a flexible parametric model described in *Methods*. The model describes the change in the mean duration as a function of viral load and also allows for variability in the duration, given a value of the set-point viral load. The best-fit model is shown in Fig. 2. This demonstrates a pattern of decline in the duration of asymptomatic infection with increasing viral load with, as expected, more uncertainty in the estimates for atypically high or low viral loads. Estimates of the mean duration of asymptomatic infection range from 15.6 years [95% confidence interval (c.i.), 9.4–31.3] for a set-point viral load of 1,000 viral copies per milliliter, through 9.7 years (95% c.i., 7.7–12.9) for 10,000 copies, 4.9 years (95% c.i., 4.1–6) for 100,000 copies, to 2.1 years (95% c.i., 0.9–3.7) for 1 million copies. Because these

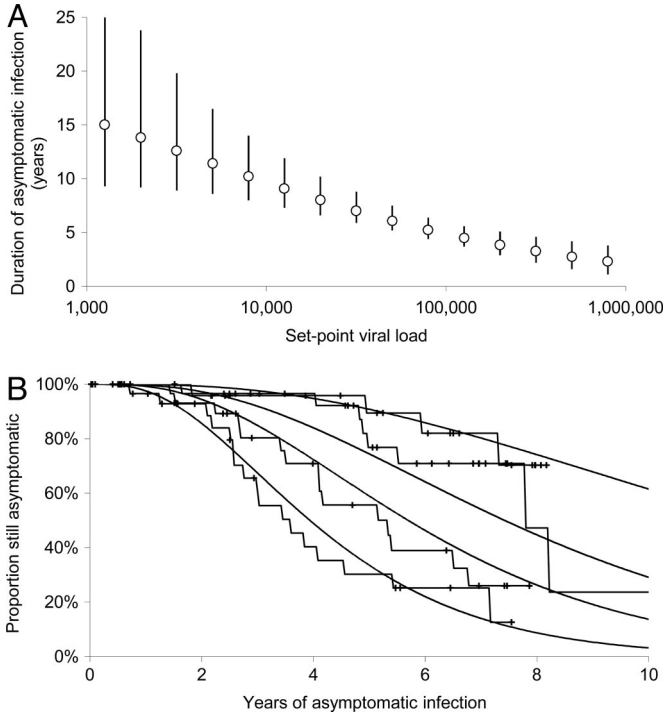

Fig. 2. Set-point viral load and duration of asymptomatic infection. The mean duration, in years, of the asymptomatic stage of infection is estimated as a function of viral load. (*A*) Best-fit and 95% confidence interval estimates are shown. (*B*) To ascertain the goodness of fit, Kaplan–Meier survival plots for the asymptomatic period are shown for patients grouped into quartiles of viral load (jagged lines, with crosses showing censored patients), along with model predictions (smooth lines).

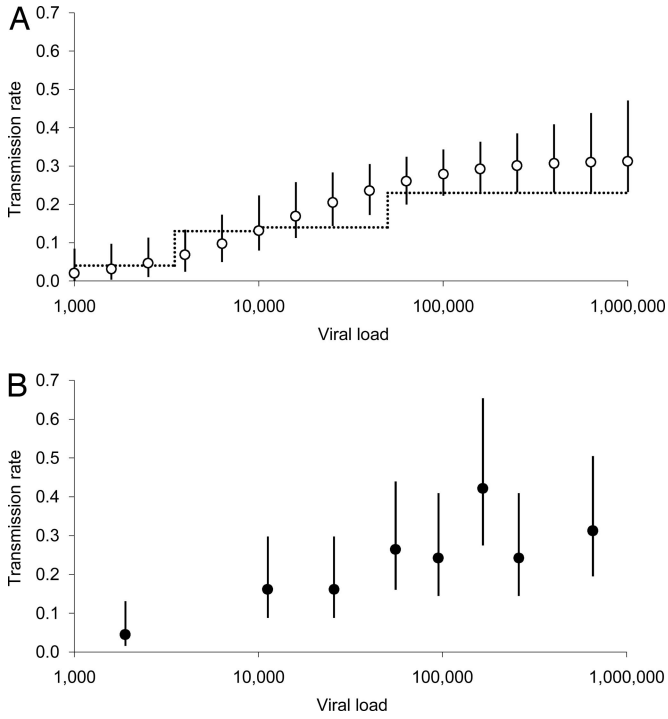

Fig. 3. Set-point viral load and infectiousness. The transmission rate per year within a stable discordant partnership is estimated as a function of viral load. (*A*) Best-fit and 95% confidence interval estimates of the transmission rate based on a parametric model fitted to the data of ref. 7. The data from the Rakai study (6) are shown for comparison (dashed line). (*B*) We also plot the transmission rate as a function of the geometric mean viral load for subjects grouped into ascending octiles of viral load for these data. This shows strong evidence for saturation of the transmission rate at high viral loads.

estimates rely on the use of parametric forms to extrapolate to extreme viral loads, we tested the use of a very general survival function, the generalized gamma distribution, but this did not fit the data significantly better ($P = 0.95$). We also considered allowing for the possibility that the mean duration could plateau to a low nonzero value at high viral loads, but this also failed to significantly improve the fit ($P = 0.18$) [see *Methods* and *Methods* in supporting information (SI) *Text*].

Infectiousness and Viral Load. Several studies have empirically estimated the rates of HIV transmission in stable heterosexual partnerships as a function of HIV-1 load (6, 7). Because these studies have focused on demonstrating the significance of the association rather than the functional relationship, we reanalyzed the data from the Zambian study (7), using a flexible parametric function to describe the dependence of the annual transmission rate within a partnership on viral load. The best-fit model, along with 95% confidence intervals, is plotted in Fig. 3*A*. Notably, we find that the transmission rate tends to reach a plateau at high viral loads. To confirm that this was not an artifact of our parametric assumptions, we plot the directly inferred transmission rate for eight groups of subjects classed in increasing octiles of viral load in Fig. 3*B*, where it is clearly seen that there is no trend for increasing transmission between the top five octiles. Estimates of the annualized transmission rate within a stable long-term discordant partnership range from 0.02 year^{-1} (95% c.i., 0.001–0.084) for a set-point viral load of 1,000 viral copies per milliliter, through 0.132 year^{-1} (95% c.i., 0.08–0.223) for 10,000 copies, 0.279 year^{-1} (95% c.i., 0.223–0.343) for 100,000 copies, to 0.313 year^{-1} (95% c.i., 0.233–0.471) for 1 million copies. Fig. 3*A* also shows the transmission rate inferred

from a cohort of HIV serodiscordant subjects in Rakai, Uganda (6), which are consistent with these estimates. Both these studies (6, 7) involved a degree of counseling to reduce unprotected sex, so transmission rates within uncounseled partnerships could be somewhat higher, although the dependence on viral load would be similar. These two studies of transmission also recorded very different rates of unprotected sex despite observing similar transmission rates, an observation that motivated our choice of focusing on the transmission rate per unit time rather than the more conventional but apparently less reliable choice of reporting the transmission probability per unprotected sex act.

Transmission Potential. One way of summarizing the epidemiological contribution of individuals with different set-point viral loads is to estimate the expected number of people infected over their entire infectious lifespan. Many factors can affect this, including host behavior, coinfections, and the state of epidemic itself, because opportunities for transmission are reduced when prevalence is already high. We define a quantity, which we call the transmission potential, as the average number of people potentially infected over the duration of the whole infectious period, in circumstances where most people are uninfected, for an infected individual with a particular viral load; where the rate of partner change is sufficiently high that it does not limit transmission, and where the transmission rate within partnerships is similar to that reported by the two cohorts studied here. We relax the second, simplifying but not crucial assumption regarding the partner change rate in *Methods* in *SI Text*. We average over all cofactors affecting transmission apart from set-point viral load. The transmission potential relates to the better known basic reproduction number R_0 discussed below, which is obtained by specifying a sexual mixing model and averaging over the distribution of set-point viral loads.

A particular concern at this stage is that we have analyzed data on the duration of asymptomatic infection as a function of viral load from a population of Dutch homosexual men infected with HIV-1 subtype B, whereas we have considered data on infectiousness from two populations of Zambian and Ugandan heterosexual individuals infected with mixed subtypes of virus. However, there is some evidence that the relation between viral load and duration of asymptomatic infection is relatively independent of subtype, population, or setting. One study of seroconverting women in Uganda found that the time to AIDS (WHO stage 4) and survival was similar to that seen in developed country cohorts (11), whereas the prevalence of general symptoms that may or may not be attributable to HIV infection (defined as WHO stage 2 and 3 events) is much higher (12). In SI Fig. 6, we show a direct side-by-side comparison of survival rates between the Amsterdam seroconverters analyzed here and a cohort of untreated female commercial sex workers in Nairobi, Kenya, followed since seroconversion (10); these show similar survival rates for individuals in similar viral load classes. In situations where this equivalence between populations and settings holds, our method provides a good estimate of the transmission potential for untreated infection in a heterosexual African population (the dominant infected population, globally).

The contribution of the asymptomatic infection stage of infection to the transmission potential depends on set-point viral load and is the product of the transmission rate and the duration of infection, plotted in Fig. 4. At low viral loads, the transmission potential is limited by low infectiousness, whereas at high viral loads where infectiousness is maximized, the transmission potential is limited by the short duration of the infectious period. Key to this shape is the observation that infectiousness does not appear to increase rapidly for viral loads over ≈100,000 copies per milliliter. The inference is robust to variation in the choice of parametric model used for estimation (SI Fig. 7).

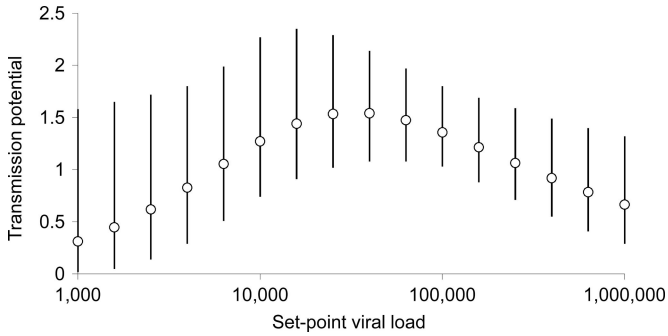

Fig. 4. Transmission potential. The transmission potential is defined as the expected number of people one case could infect over the whole course of asymptomatic infection, based on random contacts with susceptible individuals. It is the product of the transmission rate and the mean duration of the asymptomatic period (Figs. 2*A* and 3*B*) and is shown plotted with 95% confidence intervals as a function of viral load.

The periods of highest viral load are found at the start of infection, during a brief period of uncontrolled replication before the host immune system gains temporary control and, later, during the development of AIDS. These are also the periods at which the host will be most infectious (9). However, these periods are very short, and, within them, viral loads differ little between hosts, whereas there is great heterogeneity in set-point viral load. Therefore whatever the contribution of primary and end-stage infection to transmission potential (explored in detail in T.D.H., R. M. Anderson, and C.F., unpublished work), it is a baseline on top of which the major differences in transmission potential among individual hosts are determined by set-point viral load. The transmission potential for these stages is 0.67 (0.32–1.23 95% c. i.) for primary infection and 0.50 (0.31–0.96 95% c. i.) for pre-AIDS/AIDS. However, the assumption that partner change is frequent enough for it not to be a limiting factor is not likely to remain valid during these short periods of high infectiousness, and thus the transmission potential of these stages is less likely to be realized than the transmission potential of asymptomatic infection. Estimates for a variety of parameters in a simple "serial monogamy" scenario are explored in SI Figs. 9*A* and 10*A*.

Discussion

Strengths and Frailties of the Transmission Potential Analysis. We analyzed large, well studied cohorts using robust statistical methods. The models used for inference are sufficiently flexible that analyses are unlikely to be too dependent on the precise parametric forms chosen (see *Methods* in *SI Text* for some sensitivity analysis). The main assumption, that both infectiousness and duration of infection are, respectively, increasing and decreasing monotonic functions of viral load, is well supported and biologically plausible.

A significant limitation of our analysis that could be addressed in future work is that we had only data available from different sources for estimating the different parameters. We noted that the relation between the duration of asymptomatic infection and set-point viral load is similar in some different settings (10–12), but this may not be universal. For example, one study reported rapid disease progression in subtype D infections not explained by higher than expected viral loads.‖ A further concern is the

‖Laeyendecker, O., Li, X., Arroyo, M., McCutchan, F., Gray, R., Wawer, M., Serwadda, D., Nalugoda, F., Kigozi, G., Quinn, T., *et al.* (2006) *The Effect of HIV Subtype on Rapid Disease Progression in Rakai, Uganda*, Abstract 44LB, 13th Conference on Retroviruses and Opportunistic Infections, February 5–8, 2006, Denver, CO, www.retroconference.org/2006, accessed April 27, 2007.

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possible covariance between duration of infection and infectiousness, which would imply that the transmission potential, defined as the average of the product of duration and infectiousness, might not be well approximated by the product of the averages estimated here. Such a situation could arise because of joint dependence on cofactors other than viral load. Dependence on factors that independently affect survival or infectiousness, such as the dependence of infectiousness on sexual risk behavior, would not be problematic. Resolution of these concerns could be addressed by direct estimation of the transmission potential within a single patient cohort.

Consequences of Variation in Set-Point Viral Load for Public Health. Comparison of Figs. 1 and 4 shows that individuals with common, intermediate viral loads have the largest transmission potential. For current public health initiatives based on the mass deployment of antiretroviral therapy, we suggest that to attempt to maximize indirect population benefits by singling out those with the highest viral loads for treatment would be misguided (a strategy explored but not advocated in ref. 13), because it is actually the majority of patients with intermediate viral loads who ultimately cause the most infections. Although indirect population benefits of mass therapy are possible and desirable, treatment protocols in areas of limited resource should use other inclusion criteria, such as clinical need, likelihood of treatment adherence, or sexual behavior.

As for future mass interventions based on, for example, imperfect vaccines, immunotherapy, or microbicides, this framework offers a simple tool for predicting so-called "perverse outcomes" (14, 15). If the intervention reduces patients' viral load in such a way as to increase their transmission potential on average, then incidence will increase, not decrease. An intervention that reduces viral loads from high to intermediate levels and is therefore beneficial to the individual may nevertheless increase overall incidence and thus cause more overall harm than benefit. The ultimate outcome of any intervention that changes the distribution of viral loads can be predicted by calculating the change in the mean transmission potential. These conclusions are based on the epidemiological analysis of variation in set-point viral load and are independent of the evolutionary discussion that follows.

A Hypothesis: The Evolution of HIV-1 Virulence. The viral load that maximizes the transmission potential is 4.52 $\log_{10}$ copies per milliliter (Fig. 4), close to the observed means of 4.36 and 4.74 for the Dutch and Zambian cohorts, respectively (Fig. 1). Viral loads during the asymptomatic period are clustered around values that maximize the transmission potential of the virus. Is it possible that this is not coincidence but, rather, an outcome of natural selection acting on HIV-1 to maximize opportunities for onwards transmission? This would suggest that HIV-1 conforms to the classical adaptive virulence model: Seen from the perspective of the virus, a negative correlation between infectiousness and duration of infection could be interpreted as a trade-off between two viral life-history traits, with natural selection leading to an optimal balance in this trade-off (16, 17).

This adaptive virulence model for HIV-1 results in a number of clear predictions that could be regarded as tests of the hypothesis. First, for the hypothesis to be true, the observed distribution of viral loads needs to be consistent with an evolutionary interpretation of the life-history tradeoff in the transmission potential. Our analysis supports this. Second, the hypothesis predicts that set-point viral loads in transmitter and recipient will be correlated. If a trait is heritable, the conclusion that natural selection can act on it follows automatically. Conversely, if this is not the case, it is impossible for natural selection to act on a trait, no matter what its relationship to fitness might be.

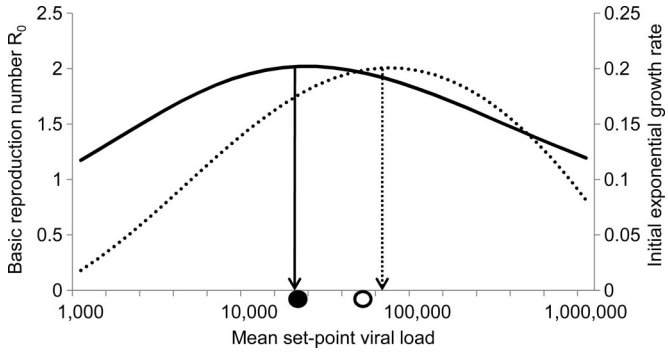

Fig. 5. Optimal viral loads in a simple transmission model. The basic reproduction number R_0 (solid line) and the initial epidemic exponential growth rate (dashed line) are estimated as functions of the mean set-point viral load of a hypothetical viral "strain." The viral load values that maximize these quantities are shown as arrows, whereas the observed mean viral loads for the cohorts are shown as circles (open for Zambia and filled for Amsterdam Seroconverters).

No studies have satisfactorily addressed the question of heritability in viral load to date. What evidence there is, direct and indirect in terms of other indicators of heritability in viral traits, is reviewed in *Discussion* in *SI Text*. We leave this as an open, testable prediction generated by our study.

Although there is no consensus on the dominant mechanisms of HIV pathogenesis, attention has shifted from within-host viral evolution (e.g., ref. 18) to pathological host immune activation (e.g., refs. 19 and 20). Viral load has been implicated as a measure of viral replication, which itself may regulate the rate of cell destruction, and mathematical models can capture the relation between set-point load and duration of the incubation period of AIDS (e.g., ref. 21). The adaptive-virulence model does not negate the role of host immune or environmental interactions in driving progression to disease but, rather, predicts that viral genetic factors modulate this progression, leading to marginally more or less severe distributions of outcomes in populations.

More specifically, the model predicts that the viral population will eventually become dominated by the "strain" with the largest basic reproduction number R_0, defined as the number of individuals infected by a typical index case in a totally susceptible population (16, 17). However, in an emerging epidemic, some strains could initially spread faster before being replaced by others with higher R_0 [so-called *r*-selection (22)]. We attempt to disentangle these processes as follows.

Consider a transmission model with multiple hypothetical viral strains. Each strain is characterized by a distribution of set-point viral loads (representing the effects of host, environmental and chance variability), but some strains have an inherited tendency to produce slightly higher or lower viral loads, on average. We use an "age-of-infection" framework (23) to calculate the basic reproduction number, R_0, and the initial exponential rate of spread, r_0, of strains characterized by their mean set-point viral load. The details of the calculation, of assumed sexual mixing, and sensitivity to the parameters are presented in *Methods* in *SI Text*. R_0 is maximized for a mean set-point viral load of 4.34 $\log_{10}$ copies per milliliter, whereas the exponential growth rate r_0 is maximized for a mean set-point viral load of 4.83 $\log_{10}$ copies per milliliter. These predicted "optimal" values are close to the mean viral loads observed in the cohorts (Fig. 5).

Because both the negative relation between viral load and duration of infection and the positive relation between viral load and infectiousness can be understood in simple biological terms, the tradeoff between these in producing the peaked transmission potential curve does not, *per se*, suggest viral adaptation. How-

ever, viral adaptation does provide a natural explanation for the relatively good agreement between the calculated optimal and observed distribution of viral loads.

An area requiring further development is the study of integrated models for exploring multilevel selection for understanding the differential roles of selection for viral replication at the cell-cell level (within the host) and host-host level (involving transmission). Although it may be thought that high viral turnover and mutation rate would favor within-host adaptation [so-called short-sighted evolution (24, 25)], these factors do not seem, in practice, to lead to any erosion of infectiousness during HIV-1 infection (9). There is also a need to explain the diversity of virus–host patterns for lentiviruses, to identify the determinants of virulence in related lentiviruses (such as HIV-2 and the simian (SIV) ancestors of HIV-1 and HIV-2 in chimpanzees and sooty mangabeys, respectively), and their relation to infectiousness and survival. A more detailed discussion of these challenges is included in *Discussion* in *SI Text*.

Conclusions

To summarize, we have quantified the transmission potential of HIV-1 as a function of set-point viral load and have found that the most common set-point viral loads result in nearly optimal transmissibility over the lifetime of the host. Crucial to these analyses were the availability of good long-term longitudinal data and the use of robust statistical methods to parameterize the dependence of infectiousness and duration of infection on viral load. The analyses should be repeated within a single cohort and in different settings. We have hypothesized that this situation could have arisen because of adaptive evolution of HIV-1 to maximize transmission between humans, although the agreement between observed viral loads and the maximum of the transmission potential could also, of course, be an interesting coincidence. The phenomenon of adaptive virulence, if verified, would have practical consequences in terms of the potential for public health interventions to impact on virulence (26). There may be as yet unidentified viral genetic factors that modulate the severity of infection.

We have explored this evolutionary hypothesis, developed testable predictions, and highlighted conceptual challenges. Testing for the existence of differences in viral load or virulence between populations and testing whether viral load is a trait heritable from one infection to the next are questions that could be answered with simple study designs. More detailed predictions and tests could be devised with dynamical epidemic models of HIV evolution. A specific challenge is predicting the time scale and outcome of natural selection acting in conflicting directions for within- and between-host viral replication. The identification of human genetic factors that determine the severity of HIV-1 infection has caused much excitement, and, to date. human genes have been shown to account for ≈10% of variability in disease progression rates (27). This leaves considerable scope for identifying other sources of variation, of which viral genetic factors have been underexplored.

Methods

The Amsterdam Seroconverters Cohort. Patients were recruited from 11 January 1982 onwards and followed at quarterly intervals thereafter (5). To minimize biases associated with the use of treatment, we included only data collected before 22 November 1993, when protease inhibitors were first introduced, resulting in a sample size $n = 123$.

Viral load was measured by quantitative PCR from frozen sera. To avoid samples collected during primary infection, measurements taken for the first 6 months after first seropositive sample were excluded, as were viral load measurements taken after the first AIDS-defining event (CDC type C). Set-point viral load was determined as the geometric mean of these measurements.

Asymptomatic Duration as a Function of Viral Load. We start by proposing the following decreasing Hill function for the duration of the asymptomatic period $D(V)$ as a function of the set-point viral load V, such that $D(V) = D_{\max}(D_{50})^{D_k}/[V^{D_k} + (D_{50})^{D_k}]$, where $D_{\max}$ is the maximum duration in years, D_{50} is the viral load at which the duration is half its maximum, and D_k is the steepness of the decrease in duration as a function of viral load (Hill coefficient).

To estimate the full profile of durations, we proposed that the probability a person is still asymptomatic at time T after primary infection is given by a gamma distribution with mean $D(V)$ and shape parameter ρ. The cumulative "survival" probability is $S(V, T) = \gamma(\rho, \rho T/D(V))/\Gamma(\rho)$, where Γ denotes the standard gamma function and γ the lower, incomplete gamma function.

Our data consists of set-point viral loads v_i for patients who progressed to AIDS or were censored after a time t_i, taken to start 6 months after the first positive test, to exclude primary infection. An indicator variable I_i is defined such that $I_i = 0$ if the patient was censored and $I_i = 1$ if the patient developed AIDS. The log-likelihood for this survival analysis is $\Sigma_i[I_i \ln[S(v_i, t_i)] + (1 - I_i)\ln[-S'(v_i, t_i)]]$, where $S'(V, T)$ is the probability density function corresponding to S.

The parameter values which maximize this likelihood are $D_{\max} = 25.4$ years, $D_{50} = 3{,}058$ copies per milliliter peripheral blood, $D_k = 0.41$ and $\rho = 3.46$. Confidence intervals for the duration were estimated at a specific viral load, V^* say, by treating $D(V^*)$ as a parameter, recasting $D_{\max}$ as a function of this and by using the likelihood ratio method to determine 95% c.i.s for $D(V^*)$. The procedure was iterated for values of V^* over a range, as shown in Fig. 2*A*. We consider the effect of using other parametric models in SI Fig. 7.

Infectiousness as a Function of Viral Load. Because of substantial inconsistencies in the reported frequency of unprotected sex acts (6, 7, 28) despite consistent seroconversion rates (Fig. 3*A*), we decided to formulate our model of infectiousness in terms of an infection hazard (probability per unit of time or rate) rather than as a probability per unprotected sex act. We thus introduced an increasing Hill function for infectiousness $\beta(V)$ a function of viral load V, $\beta(V) = \beta_{\max}V^{\beta_k}/[V^{\beta_k} + (\beta_{50})^{\beta_k}]$, where $\beta_{\max}$ is the maximum infection rate per annum, β_{50} is the viral load at which infectiousness is half its maximum, and β_k is the steepness of the increase in infectiousness as a function of viral load. The probability $p(T, V)$ that a person is infected after a time T of exposure to an infected person is $p(T, V) = 1 - \exp(-\beta(V)T)$.

Given our data (from the Zambian transmission study), consisting of set-point viral loads v_i for index cases in couples observed for a mean duration Δ, and an indicator variable I_i defined such that $I_i = 0$ if the partner was not infected and $I_i = 1$ if the partner was infected, then the log-likelihood is $\Sigma_i[I_i \ln[p(\Delta, v_i)] + (1 - I_i)\ln[1 - p(\Delta, v_i)]]$. Ideally we would have the duration of observation of each couple, but these data were not made available to us. We verified by simulation that this was unlikely to introduce systematic biases in our estimate.

The parameter values which maximize this likelihood are $\beta_{\max} = 0.317$ per year, $\beta_{50} = 13{,}938$ copies per milliliter of peripheral blood and $\beta_k = 1.02$. Confidence intervals were estimated as for $D(V)$ above.

We also considered a more general formula allowing for a minimum infection rate $\beta_{\min}$, i.e., $\beta(V) = \beta_{\min} + (\beta_{\max} - \beta_{\min})V^{\beta_k}/[V^{\beta_k} + (\beta_{50})^{\beta_k}]$, but this did not improve the quality of fit ($p = 0.5$ by one-sided likelihood ratio, the best-fit value was $\beta_{\min} = 0$).

Fideli *et al.* (7) separate the Zambian data between male and female index cases, and we repeated our analysis allowing for separate parameters for male-to-female and female-to-male

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transmission. Although the best-fit curves looked different (with a higher Hill coefficient for female-to-male transmission), the model did not fit significantly better ($P = 0.67$ based on likelihood ratio).

Transmission Potential. The transmission potential $TP(V)$ is defined in the main text as the product of the infection rate during asymptomatic infection and the duration of asymptomatic infection, i.e., $TP(V) = \beta(V)D(V)$. Confidence intervals for the transmission potential were estimated as above.

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