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Virulence and pathogenesis of HIV-1 infection: an evolutionary perspective

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Abstract

Why some individuals develop AIDS rapidly whereas others remain healthy without treatment for many years remains a central question of HIV research. An evolutionary perspective reveals an apparent conflict between two levels of selection on the virus. On the one hand, there is rapid evolution of the virus in the host, and on the other, new observations indicate the existence of virus factors that affect the virulence of infection whose influence persists over years in infected individuals and across transmission events. Here, we review recent evidence that shows that viral genetic factors play a larger role in modulating disease severity than anticipated. We propose conceptual models that reconcile adaptive evolution at both levels of selection. Evolutionary analysis provides new insight into HIV pathogenesis.

The past few years have seen impressive progress in HIV research, particularly in prevention, including the definition and characterization of broadly neutralizing antibodies for vaccine design (1), demonstration that antiretroviral treatment almost fully blocks transmission (2) and trials of pre-exposure prophylaxis that have met some success (3). Nevertheless, our understanding of pathogenesis - of why some patients progress to AIDS or need treatment quickly, whereas others remain AIDS-free for many years - remains largely unknown (4). In this review, we explore how evolutionary theory grants us a fresh perspective that leads to unexpected insights into HIV biology.

Viral load is the density of virus in someone's blood and by proxy in the rest of their body (5), and is relatively stable during asymptomatic infection, fluctuating around a value known as set-point viral load (SPVL) (Fig. 1A). A particularly important task in HIV pathogenesis research is to explain the orders-of-magnitude variation in SPVL. SPVL ranges from as low

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as <20 virions per mL of blood up to 10^6 (Fig. 1B, (5–7)), and correlates with virulence and infectiousness (Fig. 1C). Of the surrogate markers of infection severity, or virulence, SPVL is the most robust and widely used (5). Virulence is defined in untreated asymptomatic infection, as the speed of progression from infection to AIDS, by which point CD4⁺ T-cells are depleted, immunity is exhausted, and disease becomes apparent.

To date, most research on explaining variation in SPVL has focused on host factors, and particularly on components of the human immune response. Genome-wide associations studies (GWAS) have identified more than 300 single nucleotide polymorphisms (SNPs) in HLA-Class I genes (which implicate the T-cell response), and none elsewhere (8, 9), thus confirming earlier findings in the area (10). Other non-SNP host-genetic modifiers of SPVL include the -32 deletion in the CCR5 co-receptor (which reduces the ability of CCR5 tropic strains of the virus to infect cells) (11), and copy number variation in killer cell immunoglobulin-like receptor (KIR) genes (which implicate the innate immune response) (12). Despite the groundbreaking nature of these studies, the total proportion of variance in SPVL explained by host factors is limited to about 13% (increasing to 22% when adding age and sex as explanatory variables) (8).

In parallel to this research, new data and analyses suggest that viral factors are as important as host factors, if not more so, in determining disease severity. Here, we review the evidence for the importance of viral factors, as quantified by heritability of SPVL (see Box 1 and below for definition), discuss how an evolutionary perspective may help us identify these factors and generate new insights into the mechanisms of HIV pathogenesis. We suggest that the influence of viral factors on SPVL, and consequently on disease severity, have frequently been under-estimated.

Viral virulence factors

Heritability is defined as the proportion of variance in a phenotype that is attributable to the variation in underlying genotype (13). For HIV-1 SPVL, it is defined as the proportion of variance in SPVL that is attributable to viral genetic factors, which we term viral virulence factors since they influence the severity of untreated infection. For an infection trait such as set-point viral load (SPVL) heritability is closely related to (but not equivalent to) the relationship between the trait value in the transmitter (donor) host and in the recipient host within a transmission pair. The higher the heritability of SPVL, the more SPVL will be similar between the individuals in the transmission pair.

There is currently a controversy in the field concerning the magnitude of heritability of SPVL (2–9). At the high end, Alizon et al. (2) reported heritability estimates exceeding 50%. At the low end, Yue et al. (9) stated that an “analysis of 195 transmission pairs from Lusaka, Zambia revealed that viral load in the transmitting source partner contributed only 2% of the variance in seroconverter early set-point viral load ($p = 0.046$ by univariable analysis).” We demonstrate in (14) that this variation of an order of magnitude (2–50%) between these estimates does not arise from major differences between patient cohorts or any computational issues, but rather from a lack of consistency in the definition of heritability, and confusion about its indirect relation to similarity in viral load in

transmission pairs. Using a consistent framework, heritability of SPVL in heterosexual couples in sub-Saharan Africa is estimated to be 33% (95% confidence interval 22% to 45%) and is similar across studies (see Box1). These re-interpreted estimates are also broadly consistent with the results of phylogenetic methods to estimate heritability of SPVL (15, 16), though estimates obtained in this manner have been more dispersed.

Given the potential importance of viral genotype in determining SPVL, we find it surprising that we do not yet have a firm idea of what the virus virulence factors that affect SPVL are, the mechanisms by which they act, nor how they are preserved from one infection to the next, despite potentially thousands of rounds of replication and ongoing evolution within the host. However, the two most likely candidates are viral genetic features that modulate the replicative capacity of the virus, and those that influence its capacity to induce immune activation.

We consider the replicative capacity (RC) of the virus to be a likely correlate of viral virulence (23–26). RC is defined as the mean number of cells infected by a typical infected cell (directly or by viral production and infection) with plentiful access to target cells and in the absence of effective immune responses. In practice, though defined *in vivo*, it is usually measured in *in vitro* fitness assays where by definition immune responses are absent. To study the clinical relevance of RC, Kouyos and colleagues predicted RCs from viral sequences based on a complex statistical model trained on many *in vitro* measurements, and then demonstrated correlation with SPVL (24, 27).

Another study reported that the main feature distinguishing ‘elite controllers’ (i.e., individuals who do not progress to AIDS despite decades without treatment) was that they were infected by very unfit viruses, with low RC (26). It appears that the low fitness of the initial infecting viruses was the only factor the elite controllers had in common. Mutations found in some of the low fitness viruses included drug resistance mutations and escape mutations from HLA-B57 and homologs (HLA-B57 is one of the human genotypes most consistently associated with slow progression to AIDS). Escape mutations with low RC are likely to evolve within a host when the selection pressure exerted by the immune system is stronger than intrinsic selection for increasing RC. However, only 4 out of 20 elite controllers in (26) carried HLA-B57 genes or homologs whilst 6 out of 20 acquired HLA-B57 escape mutations from their infectors. Another study showed that viral escape mutations from protective HLA reduce SPVL, not just in the patient carrying the protective HLA, but also those who are infected by these individuals and who do not harbor that particular HLA type (28). Interestingly, even when these unfit viruses acquired compensatory mutations during the course of an infection that increase RC, an increase in viral load was not always observed (26). This latter finding, that later reversion of costly mutations present in early infection does not lead to increased viral load, can be explained if events taking place early in an infection are disproportionately important determinants of SPVL, since it is initial infection with an unfit low RC virus, rather than maintaining this viral genotype during the whole course of infection, which results in a low SPVL.

The second category of viral virulence determinants that might influence viral load are factors inducing CD4⁺ T-cell activation, as activation of CD4 cells is a prerequisite for

rendering cells permissive to infection (29, 30). Most prominently *nef*(31), but also *vpr* (32), *tat* (33) and *env* (34) have been implicated in modulating T cell activation. As CD4⁺ T-cell activation correlates with viral load (35), these viral genes may have an effect on viral load.

Trade-offs and population-level evolution of virulence

Not only is viral load the most widely used prognostic indicator of disease progression, but it is also positively correlated with infectiousness (Fig. 1C, (7)). This results in an evolutionary trade-off for the virus such that if there is a low SPVL, there will be more time for onward transmission before the host dies, but the probability of transmission per contact will be low. If a higher SPVL establishes then there will be less time for onward transmission, but the probability of transmission per contact will be higher. In the face of such a trade-off, natural selection should favor pathogen strains that maximize the number of onward transmission per infection, i.e. the basic reproduction number R_0 (7, 36–38).

If, as we have argued, HIV virulence factors exist, these are heritable and are partially transmitted from one infected person to the next, and if in addition these factors affect viral fitness at the between-host level, a natural corollary is that HIV virulence should be subject to natural selection at the population level. If strains differ in virulence, and this translates to differences in R_0 , then evolution will tend to select strains with the highest R_0 .

In 2007, Fraser et al (7) proposed that HIV-1 SPVL is a life-history trait that has evolved to maximize the number of transmission events. This is quantified by the transmission potential (Fig. 1D) which is the number of people one infected individual with a given SPVL might infect on average during their asymptomatic lifespan. Crucially, the distribution of set-point viral loads is clustered around values that maximize the transmission potential (compare Figures 1B and 1D) suggesting that the virus has evolved to maximize its transmission potential in untreated infection. Repeating the analysis with R_0 rather than transmission potential is more complicated, since estimation of R_0 requires assumptions about the distribution of SPVL generated by a strain, the dynamics of the sexual network and should include transmission during early and late stages of infection in addition to chronic asymptomatic infection. However, such a calculation leads to the same conclusion, that HIV has evolved to maximize the number of onward infections (7).

Conceptual models reconciling HIV adaptation at multiple levels

Within-host evolution of HIV is a rapid and dominant part of the viral life-cycle (Fig. 2A). The virus can complete a full round of replication in an infected host in less than two days (39), is under constant immune selection, and can evolve drug resistance in weeks (40). In view of ample evidence for high turnover and rapid viral evolution in the host, the finding reviewed in Box 1, that viral influence over SPVL may be partially preserved from one infection to the next, is unexpected. Evolution to maximize population level transmission potential is even more surprising. From an evolutionary perspective, we would naively expect evolution to induce adaptation at the level most proximal to viral replication, i.e. within the host, a process sometimes termed ‘short-sighted’ evolution (41, 42). We might

expect most adaptation to be targeted at optimal replication in local tissues, with no correlation of viral loads between transmitting individuals rather than the adaptation to maximize the transmission potential of the virus suggested by Figure 1. Nonetheless, there is now sufficient empirical evidence that HIV heritability needs to be explained, and therefore that mechanisms of HIV pathogenesis need to be compatible with multilevel selection, allowing the virus to adapt to both proximal within-host and distant between-host selection pressures. This compatibility requirement is a strong constraint. We propose three possible (and not mutually exclusive) mechanisms which meet this constraint in Fig. 2B-D.

Mechanism 1: Slow evolution of replicative capacity

We know that viral turnover and mutation rate are both high, but this does not necessarily imply that all viral phenotypes, such as virulence traits, should evolve rapidly (Figure 2B). First, the within-host fitness landscape may be highly complex and rugged (27, 43), which might slow within-host evolution and adaptation. Second, viral strains with a high *in vitro* replicative capacity might not reach sustained high frequencies within an individual if they are intrinsically more immunogenic, or because they are preferentially targeted by acquired immunity as a result of their dominance during early infection. For example, one common consequence of immune selection is the emergence of mutations that allow the virus to escape cytotoxic T-lymphocyte (CTL) responses. Many CTL escape mutations reduce the *in vitro* replicative capacity of the virus, but are nonetheless under intense positive selection during the course of infection because they help the virus evade the host immune response (44). Compensatory mutations can restore fitness after escape, but evolution through a process of continuous immune escape and compensatory evolution may slow the overall pace of evolution of net replicative fitness, and would shift the balance of selection towards population level selection.

Mechanism 2: Little within-host selection for viral load

It is conceivable that there are viral factors that strongly affect viral load but are neutral with respect to within-host fitness, and thus do not evolve rapidly within a host (Fig. 2C, (45)). Owing to the absence of strong within-host selection, such factors would result in the heritability of virulence and the evolution of viral loads that maximize the between-host transmission potential. Using a generic model of HIV replication, Bonhoeffer et al. (6) proposed that differences in the rate of activation of target cells may be a major source of variation in virus load. Though it can replicate in many cell types HIV replicates most efficiently in activated CD4+ T-cells (30). If the virus activates target cells systemically, then any viral strain that activates cells provides a replication benefit that is not exclusive to just this strain but rather to all competing strains in the body, to the extent that all are provided with additional target cells. Thus, virus-induced activation of target cells can be regarded as contributing towards a “public good” (20), and there would be no selective advantage for a viral strain that increases the production of target cells. Which viral factor fulfills the required criteria for virus-induced and systemic target cell activation remains unclear. However, *nef* is clearly an interesting candidate. Firstly, one of the *nef*-linked pathways of T-cell activation may indeed be systemic as it is mediated through the release of soluble

factors secreted by *nef*-expressing macrophages (31, 46). Secondly, patients with *nef*-deficient virus have low viral load (47, 48).

Mechanism 3: Influence and transmission of founder viral strains

Another explanation for heritability of SPVL and evolution of HIV to maximize transmission could be preferential transmission of viruses that have not undergone many rounds of replication in the host, either because transmission occurs during early infection (49), and/or because of the transmission of viruses that have been sequestered in long-lived cells (a process referred to as ‘store and retrieve’ (50)). This process, coupled with the disproportionate influence of the genotype of viruses from early infection on SPVL would essentially make any within-host adaptation an evolutionary dead-end for the virus (Fig. 2D). The viral response to multilevel selection would thus be akin to differentiation made between the germ-line and soma, that could itself be an adaptive strategy of the virus to maintain transmissibility. During the course of HIV infection, a small fraction of infected CD4⁺ T cells transition into long-lived memory CD4⁺ cells containing an integrated copy of the viral genome, thus creating a long-lived reservoir of stored virus (51). Since much of this sequestration occurs during the early stages of infection (52) when viral loads are greatest, the reservoir acts as an archive of early viral sequences similar to the ancestral sequence(s) that initiated the infection. Occasionally, sequestered virus is retrieved from the archive by the reactivation of a latently infected cell (51). If retrieved virus is preferentially transmitted, then the virus an individual transmits will be similar to the virus that initiated the infection (Fig. 2D). There is evidence that viruses present during acute and early infection have a transmission advantage associated with distinct genotype (53) and phenotype (54), lending support to this hypothesis. This mechanism also explains the long-standing phylogenetic puzzle of why HIV evolves faster within-hosts than it does at the epidemiological level (50, 55, 56), and is partially supported by data from deep sequencing of viruses in transmission events (57). On the other hand, that both immune escape and drug resistance mutations are on occasion transmitted (26) demonstrates that on at least some occasions it is extant, not stored viruses that are transmitted. The quantification of the preferential transmission of stored virus requires further study.

To explain evolution to maximize transmission, it is not enough that founder virus is preferentially transmitted. Its genotype also needs to influence viral loads throughout the course of infection more than variants that arise later. An example of such influence is that individuals infected with viruses with low RC tend to develop low SPVL even though the virus may increasing its RC during the course of infection (26, 28) as previously discussed. One mechanism that could generate such an association would be if the RC of the virus impacts on the severity of acute infection and in particular the extent of damage to the gut. Gut damage would exacerbate the long-term level of microbial translocation, hence promote immune activation, increase the supply of target CD4⁺ T-cells and thus enhance the set-point viral load (58). Whatever the mechanistic basis, there is some empirical evidence that acute infection severity correlates with SPVL (59, 60).

Distinguishing between the mechanisms

Each of these mechanisms is sufficient to generate heritability of SPVL and allow population-level evolution to take place, but they are not mutually exclusive. Distinguishing which of these mechanisms are at work would require a better understanding of which aspects of viral biology influence SPVL. Large-scale HIV viral whole-genome association studies, that search for correlations between viral polymorphisms and SPVL may enable us to detect which parts of the viral genome are correlated with disease severity, provided the relevant mutations or genomic motifs are common and have sufficiently strong effect. Follow-up experimental work may elucidate their mechanism of action. In addition to recapitulating well-known findings on the association between human HLA and viral genotype and SPVL, Bartha et al (61) reported a viral whole-genome association study on SPVL, which did not find any statistically significant association between virus proteome and SPVL. The study, with N=698 was however only powered to detect individual amino-acid variants that explained 4% of variation of SPVL.

Future viral whole-genome association studies will require greater sample sizes, and should include viral genetic polymorphisms and motifs in analyses. Identification of viral factors and of their mode of action will enable us to better understand pathogenesis, and to distinguish between viral replication as the primary factor influencing virulence, viral-induced immune activation, or other, as yet unknown, factors. Further testing to show whether ancestral sequences are indeed preferentially transmitted should also be an important goal of future research. This would require not only deep sequencing of viruses from donor and recipient patients close to the time of transmission, but sequencing from the donor sampled at multiple times prior to transmission. For ethical reasons, such studies would need to be retrospective, and could also be expanded to consider transmission chains. Without these data, there is no way of determining whether a transmitted virus is an early variant that previously been sequestered in long-lived cells. Studying virus sampled from different body compartments in such transmission pairs would also be valuable, as the blood compartment may be less relevant to sexual transmission than the genital mucosa or semen, for example.

Beyond the theory

Most recent research on HIV pathogenesis has focused on the host and its immune response, with more recent developments than can be summarized here (see (62) for a up-to-date collection of reviews, also available from perspectivesinmedicine.cshlp.org/cgi/collection/hiv). The aim of this review has been to reframe the debate on HIV pathogenesis to include greater emphasis on the virus and its genotype, how viral variation interacts with the host response, and how certain heritable viral characteristics influence the course of disease. It is now difficult to ignore the mounting evidence that heritable viral virulence factors exist, and that they have an important role in HIV pathogenesis. But, what they are, how they are maintained during the course of infection and from one infection to the next, despite extensive viral replication between transmission events, and how they interact with now well studied host factors, are gaping holes in our knowledge. We have presented a framework to

reconcile the potentially conflicting observations and key questions regarding HIV pathogenesis in the context of a synthesis based on evolutionary models.

In interpreting our findings, it is tempting to compare our estimate of HIV SPVL heritability (33%) to the lower estimate of 13% attributed to host genotype by GWAS, but these numbers are unfortunately not strictly comparable. GWAS tend to be conservative due to the problem of massive multi-hypothesis testing. In addition, they typically focus on 'narrow-sense' heritability, which is the sum of individual contributions to SPVL directly attributable to single SNPs in human genomes (63). In contrast, the estimate of 33% for viral effects is 'broad-sense' heritability and accounts for the total effect of viral genotype on SPVL, including possible complex interactions between viral SNPs and other genotypic motifs. As a result, broad sense heritability is always larger than narrow-sense heritability. Furthermore, if some of the variance is caused by interactions between host and virus genotype, there could be double counting in these measures. The relative role of host, virus and interactions in explaining SPVL is still not defined, but we have argued that viral virulence factors that influence SPVL have often been underestimated.

Understanding viral virulence factors will help us to predict how the virus may evolve in response to different mass public health interventions. The widespread deployment of antiretroviral treatment, one of the great public health success stories of our time, alters the transmission trade-off and could plausibly select strains of increased virulence. Set-point viral loads appear to have been increasing in some highly treated populations (64), though observations remain inconsistent (65). In predicting future changes, a further trade-off between increased virulence and drug resistance also needs to be considered, since as discussed above drug resistance appears at least in some cases attenuate the virus (26). If changing virulence is a concern, sentinel surveillance of viral load amongst treatment naïve individuals could be straightforwardly combined with drug resistance testing, and would provide sufficient data. Furthermore, increasing virulence is expected to be slow and limited, and does not challenge the current priorities of HIV treatment and prevention programs, since it only reinforces the need for prompt diagnosis and treatment. Thinking further afield, an understanding of virulence factors could inform therapeutic strategies aimed at attenuation of infection in-host, since at least in some cases of naturally attenuated infection rapid reversion of virus back to higher levels does not seem to occur (26, 28, 48).

Our focus here has been predominantly on mechanistic evolutionary models of HIV virulence as assessed in individuals infected with closely related viruses. Future work could expand this approach to explain the documented difference between subtypes of HIV where both disease and infectiousness may vary (66, 67), to explain virulence in the ancestral SIV viruses infecting a wide range of old world monkeys and primates with very variable outcomes of infection (68), and to study virulence and multilevel selection in other RNA viruses causing chronic infection, such as HTLV and HCV. Perhaps most importantly, an evolutionary perspective provides new insight into the basic mechanisms of HIV pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box**Heritability quantifies the effect of viral genotype on SPVL.**

In quantitative genetics, the heritability of a trait is the proportion of the variance in the phenotypic value observed in a population that is attributable to genotype (13). This measure indicates the importance of genetic variation in shaping phenotypic variation. Recently, this concept has been applied to infectious diseases: for HIV, the heritability of a phenotypic trait (e.g., virus load) is the proportion of variance in this trait that is attributable to viral genetic factors. Since SPVL is a feature of the natural history as a whole, heritability of SPVL is defined with respect to the ensemble of genotypes of viruses that infect and evolve within the host. The simplest study design to measure heritability in SPVL is to look at the similarity in trait values between individuals in couples where transmission is strongly suspected to have occurred (17–22). If SPVL is indeed heritable from one infection to the next, there should be similarity between a ‘recipient’ and ‘donor’ viral load. More technically, broad-sense heritability (conventionally denoted h^2) is estimated by the slope of the regression line, r , of the recipient viral load on the donor viral load (see SOM). Estimates of r obtained by independent studies have been quite consistent despite differences in their presentation due to differences of focus: few studies have presented h^2 as the key summary statistic. Estimates of h^2 , adjusted for many confounders, are shown for several studies in Box Table. An alternative design for estimating heritability, inspired by the phylogenetic comparative method, is to use virus sequence data from each host to reconstruct a phylogeny of infections and estimate the relationship between virus genetic relatedness and SPVL (Box Figure). Based on this approach, Alizon et al. (16) found that up to 59% of variance could be explained by viral genotype. Variation among heritability estimates obtained in different studies should not necessarily be taken as an expression of the uncertainty of the individual estimates since heritability is a measure that also depends on the population it is estimated in (13). For example, a very low diversity epidemic resulting from an explosive burst of transmissions would exhibit lower heritability than a high diversity mature epidemic due to low background diversity in genotypes against which to make comparisons.

Box Table 1
Summary of estimates of heritability of SPVL
from transmission couples in sub-Saharan Africa,
and overall aggregate summary estimate.

Estimates of heritability, the proportion of variance in set-point viral load that can be attributed to viral genetic factors (best estimate and 95% confidence interval). Studies reviewed here were selected as studies of transmission amongst heterosexual couples in sub-Saharan Africa.

N Couples	Heritability	Study [Reference]	Country, Subtype(s)	Adjustments
115	36% (12%-60%)	Tang et al. [17]	Zambia, mostly C	Duration of infection in seroconverter, Age, Sex, HLA
97	36% (6%-66%)	Hollingsworth et al. [18]	Uganda, mostly A, D and recombinants	Age, Sex, Subtype, Symptomatic GUD
141	44% (19%-69%)	Lingappa et al. [21]	Partners in Prevention (14 sites in East and Southern Africa), Diverse subtypes	Age, Sex, Subtype, Symptomatic STI, GUD, Circumcision, Hormonal Contraceptive Use, Source partner characteristics.
195	26% (8%-44%)	Yue et al [22]	Zambia, mostly C	Age, Sex, HLA, HLA sharing between partners.
548	33% (22%-45%)	Overall summary estimate (weighted by standard error)

Box Figure 1.

Phylogeny of HIV isolated from 134 Swiss men who have sex with men (left) with well-defined SPVL (indicated by the color of tips). Heritability is estimated by comparing phylogenetic proximity to similarity in SPVL (16).

Figure 1. the evolutionary epidemiology of viral load.

A, the typical pattern of viral load in untreated HIV-1 infection, with a very high peak during the first weeks of infection, and a gradual increase in the late stages of infection. During asymptomatic infection, viral loads are often relatively steady, fluctuating around a value known as the set-point viral load (SPVL). **B**, the distribution of SPVL in a cohort of patients infected with subtype B virus; the distribution is similar across populations and across viral subtypes (69). Note the log-scale on the x-axis, which demonstrates variability over orders of magnitude. To illustrate the extraordinary extent of this variability, inset plots show

simulated viral density in 5 μ L of peripheral blood for two values at either side of the distribution of viral load: thus viral load is a strong predictor of both infectiousness and rate of progression to disease. The distribution of SPVL was previously described with (7), and we have updated this to include SPVL data from 2,015 seroconverters in the cohort (with kind permission from Drs. de Wolf and Reiss). (**C**). Infectiousness, estimated within heterosexual couples, is shown in blue. The severity of infection, estimated as the mean time from seroconversion to AIDS, is shown in green (from (7)). Lines show best fit, and filled areas show 95% confidence intervals. **D**, the transmission potential is a summary measure of the epidemiological ‘success’ of an infection with given set-point viral load: it is the mean number of expected secondary infections over the whole chronic and asymptomatic infectious life-span, estimated as the product of infectiousness and duration of asymptomatic infection (from **C**). The close agreement in between the optimal evolutionary strategy (labeled in **D**) and the distribution of viral loads (plotted in **B**) suggested the hypothesis that the distribution of viral loads is in fact the outcome of viral adaptation to maximize the transmission potential (7). None of the calculations account for the effect of treatment. This is reasonable for of an evolutionary analysis, since treatment has unfortunately only become widely available in recent years.

Figure 2. mechanisms that could reconcile rapid within-host evolution with heritability of SPVL and adaptation to maximize transmission opportunities.

A, Illustration of a full transmission-to-transmission viral replication cycle. During or shortly after transmission, the virus experiences a very strong bottleneck (70), and expands clonally, mostly in activated CD4⁺ T-cells. Most viral replication is very fast, with a cellular life-cycle of one or two days (39, 40), but a reservoir of virus in long-lived CD4 cells is quickly established, is continuously replenished, and persists for life (52). Viral replication quickly becomes systemic, assisted by chronic and persistent immune activation, with gut-associated lymphatic tissue and germinal centres in other lymphatic tissues being particularly privileged sites of replication (52, 58). The most uncertainty in the lifecycle surrounds which viruses, if any, are preferentially transmitted to found new infections in other hosts. **(B-D)** Three mechanisms that could reconcile within-host adaptation with heritability and population-level viral evolution to maximize transmission; these mechanisms are described by schematic representations of the viral lifecycle. In mechanism 1 **(B)**, population-level evolution is explained by evolutionary constraints that slow within-host evolution of virulence traits in the host, perhaps due to virulence genotypes requiring complex combinations of mutations rather than individual non-interacting point mutations. In mechanism 2 **(C)**, population-level evolution is explained by an absence of selection within the host for viral virulence factors. In mechanism 3 **(D)**, population-level evolution is explained by separation of within-host and between-host adaptation caused by preferential transmission of viruses that are stored during early infection, together with disproportionate influence of the founder genotype on SPVL.