HIV-1 virulence, fitness and replication capacity

Eric S Daar

Harbor-UCLA Medical Center, Division of HIV Medicine, 1124 West Carson St, N-24 Torrance, CA 90502,USA
Tel: +1 310 222 2467
Fax: +1 310 533 0447
EDaar@LABioMed.org

The virulence and fitness of a given pathogen can influence the pathogenesis of disease. Recent studies have demonstrated numerous factors that contribute to the virulence and fitness of HIV-1 *in vivo* and *in vitro*. Moreover, emerging data shows that variability in these factors is likely to influence the natural history of disease and potentially be altered by antiretroviral therapy. These observations have led to further studies that have enhanced our understanding of how drug resistance mutations can alter viral fitness, which in turn may have substantial implications for those with limited treatment options. This review will define viral virulence and fitness in the context of HIV-1 infection and summarize the current data demonstrating how this may be relevant to the pathogenesis of disease and how it could potentially be exploited in the future to optimize the management of patients in the clinic.

Early in the human immunodeficieny virus (HIV) type 1 epidemic it was observed that there was extensive heterogeneity in the natural history of disease. Cohort studies initiated prior to the availability of potent antiretroviral therapy showed that the time from HIV-1 infection to the development of clinical AIDS varies from months to years, with some individuals remaining asymptomatic with normal CD4$^+$ T-lymphocytes for more than a decade. Numerous studies have explored virologic, immunologic and host factors associated with the rate of disease progression. In the last decade, host genetic factors have been clearly shown to influence the natural history of disease [1–3]. In addition, plasma HIV-1 RNA is also known to predict CD4$^+$ T-lymphocyte decline and disease progression, as has the level of CD8$^+$ T-lymphocyte activation [4–8].

Qualitative virologic markers have also been associated with the rate of disease progression. This includes the presence of virus with the biologic phenotype to infect T-cell tumor lines and induce syncytia *in vitro*, syncytium-inducing (SI) virus. The latter is now well known to be related to coreceptor tropism for CXCR4 (X4 viruses) [9–13]. There has been additional interest in evaluating the influence other qualitative virologic markers have on HIV-1 progression, in particular, novel measures of viral 'virulence' and/or 'fitness'. Research related to viral fitness has been intensified by the observations that drug resistance mutations can often render a virus less 'fit' and that this may influence HIV-1 disease progression [14–16]. In fact, this observation has been proposed as an explanation for the modest but significant viral suppression seen in those with high level drug resistance undergoing lamivudine (3TC) monotherapy [15]. More recently, alterations in viral fitness of drug resistant viruses have been hypothesized as the mechanism by which those experiencing virologic rebound on potent antiretroviral therapy maintain stable CD4$^+$ T-lymphocyte numbers [17,18]. This article will summarize current data related to viral virulence and fitness.

Viral virulence

The virulence of an infectious agent is best defined as its ability to cause end-organ damage. In the case of HIV-1 this refers to its ability to damage lymphoid tissue, *in vivo* as well as *ex vivo* in tissue culture or lymphoid extracts. Virulence of HIV-1 can also be applied to other targets of pathogenicity, such as the CNS. Along with viral-specific characteristics, *in vivo* measures of virulence are certain to be influenced by host factors and target tissue variability. An example of a situation where a highly replicative pathogen lacks virulence is the case of simian immunodeficiency virus (SIV) cpz. This virus replicates to high levels in chimpanzees without causing clinical disease [19]. In contrast, other SIV strains have been shown to be highly pathogenic, resulting in complete and irreversible depletion of CD4$^+$ T-lymphocytes and progression to clinical AIDS and death within weeks of infection [20]. Similar observations can be made *ex vivo* using SI or X4 strains of HIV-1 in peripheral blood mononuclear cell (PBMC) culture as well as lymphoid extracts [21–23].

Keywords: capacity, fitness, HIV-1, pathogenesis, replication

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Measures of *in vivo* virulence have been limited to animal models. The ability to investigate *in vitro* virulence has been constrained by a paucity of available assays that have high throughput and/or the potential to be utilized in the clinic. The best example of a virulence factor that appears to be related to HIV-1 disease progression is the relationship between the emergence of SI or X4 virus(es) and $CD4^+$ T-lymphocyte decline and progression to AIDS [13,23–25]. Although the temporal relationship between the development of these potentially more virulent strains and immunologic decline are compelling, it remains unproven that this switch in biologic phenotype or coreceptor tropism is the direct cause of progression. Moreover, even if these virologic changes were proven to be causative of clinical progression, they alone do not explain viral pathogenesis since up to 50% of those who die from AIDS do so without detectable SI or X4 virus [9]. This may in part relate to a recent observation demonstrating that despite the fact that X4 viruses are more cytopathic, they tend to replicate less efficiently than R5 viruses in primary $CD4^+$ T-lymphocytes [26]. While virulence factors need to be further explored in the context of HIV-1 immunopathogenesis, the clinical utility of these markers are likely to remain limited.

Viral fitness

HIV-1 fitness is a measure of the replicative adaptation of the particular virus to its environment. During the course of HIV-1 infection, the high replication rate and infidelity of the reverse transcriptase enzyme allows for rapid viral evolution that can result in a virus that has a replicative advantage *in vivo*. Such an advantage could be conferred by immunologic escape or the emergence of antiretroviral drug resistance. In fact, viral fitness can be considered the result of a continuous process that matches viral properties against influential factors in the *in vivo* milieu, such as evolving immune responses, target cell factors and availability, as well as antiretroviral drug pressure.

Table 1. Methods for measuring HIV-1 fitness.

Ex vivo	Catalytic activity of protease or reverse transcriptase Viral growth kinetics *in vitro* in PBMCs Growth competition in tissue culture Single-cycle recombinant assay
In vivo	Viral kinetics in plasma SCID-hu/thy murine model

PBMC: Peripheral blood mononuclear cell; SCID: Severe combined immunodeficiency.

The intrinsic ability of a given strain of HIV-1 to replicate is likely to be influenced by multiple steps in the viral life cycle from cell binding to fusion, reverse transcription, integration, transcription, translation and particle assembly, and viral budding. Fitness in the *in vivo* setting is defined by the intrinsic viral properties as well as the influence of other factors, such as immune responses and antiretroviral therapy. Consequently, *in vivo* fitness can change with alteration in important cytotoxic T-lymphocyte epitopes or targets of antiretroviral therapy such as reverse transcriptase or protease [14,27,28]. In contrast, while such mutations may enhance the ability of the virus to grow *in vivo*, such evolution could result in a strain that is substantially less 'fit' *in vitro.* This is particularly apparent in the context of drug resistance where the resistant virus predominates under drug pressure only to be replaced by wild-type virus when therapy is discontinued [29]. In order to ultimately explore the influence viral fitness has on HIV-1 immunopathogenesis, reliable measures of fitness must be developed. Once such assays are proven to be reproducible, further studies can explore their potential relationship with HIV-1 disease and only then can consideration be given to how such an assay might be utilized in the clinic to improve the care of HIV-1-infected individuals.

Measuring HIV-1 fitness

Several *in vivo*, *ex vivo* and *in vitro* assays for measuring viral fitness have been investigated (**Table 1**) [30]. Although the *in vivo* measurement of fitness are likely to be most relevant, such assays are difficult to perform and generally rely on the relative proportion of virus detected using any of a variety of molecular methods [16,31]. Experiments can also be carried out in animal models but do not account for the true *in vivo* milieu in a given individual [32,33]. *Ex vivo* assays can be performed that demonstrate the intrinsic capacity of plasma virus to replicate relative to reference strains. These measures can also be performed by examining the catalytic activity of viral enzymes [14,15,28,34–37], *in vitro* replication [15,35,38–41], or using growth competition assays with whole virus [42,43] or recombinant viruses that contain select genomic portions of the given individuals virus [44–46]. More recently, assays have been developed using single-cycle infection to assess viral infectivity [35,46–50]. Finally, a novel single-cell assay was described that used plasma-derived gag–pol sequences in a vector that allowed for flow cytometric analysis. This assay

shown to emerge in longitudinally followed subjects noted to experience precipitous declines in CD4+ T-lymphocytes [23,54].

In 2003 the author and colleagues presented data at the Interscience Conference on Antimicrobial Agents and Chemotherapy assessing the relationship between coreceptor tropism and disease progression using an envelope recombinant vector assay (**Figure 2**) [55,56]. In a cohort of longitudinally followed HIV-1-infected hemophiliacs, those with any detectable X4 virus at baseline had a lower CD4+ T-lymphocyte count, higher plasma HIV-1 RNA, and even when controlling for these factors were found to experience more rapid immunologic decline and clinical disease progression [56].

Several qualitative measures of viral fitness have been further explored in natural history studies. Quinones-Mateo and colleagues analyzed the relationship between viral fitness using an *ex vivo* dual virus competition assay in those with long term nonprogression of disease versus more rapid progressors [43]. In this study they demonstrated that virus from the rapid progressors' outcompeted virus from nonprogressors. Furthermore, they demonstrated a significant relationship between viral fitness and plasma HIV-1 RNA. Another study recently published by Barbour and colleagues assessed the relationship between *pol* RC of virus present in untreated subjects that were recently infected with HIV-1 [52]. Here they used the ViroLogic Inc. single-cycle assay and showed that there was considerable variability in RC among these antiretroviral-naive, recently infected individuals. They further demonstrated that *pol* RC correlated with baseline CD4+ T-lymphocyte number and that increasing RC values predicted immunologic decline. Campbell and colleagues also assessed the relationship between viral fitness *in vitro* and plasma HIV-1 RNA. This group characterized virus from 15 subjects not on antiretroviral therapy, culturing autologous virus in PBMCs and using a recombinant single-cycle assay that included RT and PR alleles to show that there was variability in RC values across the group, that this measure of RC correlated with other measures of replicative capacity, and with plasma HIV-1 RNA [57].

The recombinant single-cycle *pol* RC test has been developed as a reproducible, high throughput assay that allows for the retrospective analysis of well-documented cohorts to assess the relationship between this marker and HIV-1 disease progression. In a cohort of 126 minimally-treated hemophiliacs the author and colleagues recently showed that there was substantial variability of RC in an approximate normal distribution [56], as has been seen in drug susceptible virus in the ViroLogic Inc. database [58]. In addition, the author and colleagues found in a cross-section analysis that increasing RC was associated with lower CD4+ T-lymphocyte number and increased rate of C4+ T-lymphocyte decline, even when controlling for baseline CD4+ T-lymphocyte number, plasma HIV-1 RNA and coreceptor tropism. This measure also independently predicted progression to clinical AIDS. Taken together, the above studies provide evidence that the *pol* RC measure is at least associated with the rate of HIV-1 disease progression. Further interest is currently focused on how viral fitness might influence HIV-1 transmission, particularly with regards to that of drug-resistant viruses. More research is certainly needed but it is conceivable that select mutations may result in differences in viral fitness that could alter the efficiency with which HIV-1 variants may be transmitted [59]. While difficult to design conclusive studies, research in the future will focus on comparing the prevalence of select resistant virus among newly infected individuals and those of potential transmitters in the community in an attempt to address this important question.

HIV-1 fitness & drug resistance

It is increasingly clear that the antiretroviral drug-resistant virus is more fit *in vivo* when under drug selection pressure. However, mutations conferring resistance often result in an intrinsically less fit virus than the wild type when drug pressure is not present [60,61]. Early clinical evidence of this was seen in the first studies of 3TC monotherapy where, despite the rapid emergence of high level drug resistance, modest but significant levels of viral suppression were sustained [62]. Furthermore, when 3TC was discontinued there was the rapid emergence of wild-type virus. Subsequently, *in vitro* correlates of fitness confirmed that strains with 3TC resistant mutations (M184I/V) were less fit than those without [15]. Similarly, other antiretroviral drug resistant mutations have been shown to confer reductions in *in vitro* viral fitness. For example, virus with the thymidine analog mutation at codon 215 conferring zidovudine (ZDV) resistance (T215Y) appears less fit *in vitro* and often reverts to more fit variants when drug selection pressure is removed (T215C/D) [15,16]. In addition, Miller and colleagues reported data from the ViroLogic Inc. database demonstrating

the relationship between various NRTI mutation patterns and pol RC. Along with the profound effect M184I/V has on fitness, they showed that the K65R mutation, associated with broad NRTI resistance, also had markedly impaired fitness, particularly if coexisting with the M184I/V mutation [63–65]. Similar declines in fitness have been seen with various protease mutations, such as D30N and L90M [40,66]. In fact, an *in vivo* experiment demonstrated in a severe combined immunodeficiency (SCID)-hu/thy murine model the effect PI-resistance can have on the pathogenicity of HIV-1. In this case, infection with the resistant virus induced a less cytopathic effect than wild type strains of the same virus [33]. This topic is further reviewed by Nijhuis and colleagues [67].

Clinical relevance of the drug resistance associated effects on viral fitness

Early observations demonstrated that those taking 3TC monotherapy experienced sustained viral suppression despite the development of high level drug resistance. This highlighted the potential clinical relevance of drug resistance-associated changes in fitness [15,62]. These early observations were further supported by clinical data in those taking combination antiretroviral therapies that included 3TC. One recent study tested the hypothesis that the M184I/V mutation's effect on viral fitness influences viral and immunologic parameters. In this case, investigators simply discontinued 3TC in those with detectable viremia on a stable 3TC-containing regimen and showed that plasma HIV-1 RNA increased [68]. Castagna and colleagues recently reported the preliminary results of a controlled clinical trial of subjects with persistent HIV-1 viremia on a stable regimen that were randomized to either discontinue all therapy or all drugs except 3TC [69]. After 24 weeks of follow-up, the latter group was noted to maintain better HIV-1 suppression and higher $CD4^+$ T-lymphocytes compared with those that discontinued all drugs.

In the era of potent antiretroviral therapy clinicians noted that even those experiencing viral rebound on therapy maintained immunologic stability. Ledergerber and colleagues observed that people receiving PI-containing antiretroviral therapy who experienced viral rebound had a similar risk of progression to those with persistent suppression [70]. Kaufmann and colleagues made related observations in the Swiss Cohort Study, finding that despite the fact that some experienced viral rebound they maintained stable $CD4^+$ T-lymphocytes for several years [17]. One proposed hypothesis to explain these observations was that the drug-resistant virus was sufficiently impaired in its ability to replicate such that it was less cytopathic. Consistent with this was the observation that those who discontinued their treatment, regardless of whether they were virologically suppressed, experienced prompt immunologic decline [17,29].

Summary & conclusions

The qualitative measure of HIV-1 virulence and fitness are likely to in part explain heterogeneity in the natural history of disease. The best characterized of these markers has been the ability to infect T-cell tumor lines and induce syncytium (SI viruses) *in vitro* [9,12,13,71]. This phenotype has more recently been shown to be associated with tropism for the CXCR4 secondary receptor (X4 viruses) [10]. Changes in coreceptor tropism have also been shown to have pathogenic consequences on lymphoid tissue *ex vivo* and *in vitro*, as well as to correlate with increased risk of disease progression [21,22].

The replicative capacity of HIV-1 has been assessed using several different assays capable of distinguishing one isolate from another. Multiple portions of the virus have been shown in recombinant assays to contribute to the overall replicative capacity. Most recently, *pol* RC has been evaluated and shown to be variable from person to person, as well as being markedly altered in those with antiretroviral therapy-associated resistance mutations. Several studies in recently and chronically infected individuals have shown that the natural variability in this measure predicts change in $CD4^+$ T-lymphocytes and clinical disease progression [52,56]. Furthermore, impaired replication of drug resistant HIV-1 appears to be closely related to $CD4^+$ T-lymphocyte numbers in those experiencing viral rebound on antiretroviral therapy [29].

Data is continually emerging showing that currently available measures of HIV-1 virulence and fitness are clinically relevant and impact the natural history of disease. Although, the optimal method for applying these assays in clinical practice remains to be elucidated, enhancing our understanding of how different drug resistant patterns influence *pol* RC could allow for new treatment strategies for those with limited therapeutic options.

Highlights

- The natural history of human immunodeficiency virus (HIV) type 1 disease progression is highly variable.
- Variability in the natural history of disease is related to host genetic factors, immunologic responses, as well as quantitative and qualitative viral properties.
- Viral virulence of an infectious agent is best defined as its ability to cause end-organ damage; for HIV-1 this would include lymphoid tissue and the CNS.
- The best characterized virulence factors for HIV-1 have been the biologic phenotype to induce syncytia *in vitro*, a factor highly correlated with CXCR4 coreceptor tropism (X4 virus).
- Little is known as to why syncytia-induced (SI) and X4 viruses emerge and if they are the direct cause of disease progression.
- HIV-1 fitness is a measure of the replicative adaptation of the particular virus to its environment.
- Viral fitness can be considered the result of a continuous process that matches viral properties against influential factors in the *in vivo* milieu, such as evolving immune responses, target cell factors and availability, as well as antiretroviral drug pressure.
- Intrinsic viral fitness is defined by multiple steps in the viral life cycle.
- Drug-resistant mutations reduce the intrinsic ability of that virus to grow *in vitro,* while the same virus may be more 'fit' than wild type *in vivo* in the setting of antiretroviral pressure.
- HIV-1 fitness can be measured *in vivo*, *ex vivo* and *in vitro.*
- Multiple parts of the viral genome contribute to fitness.
- Measures of whole virus fitness have been correlated with disease progression.
- *Pol* replication capacity has been shown to predict disease progression in untreated and minimally antiretroviral therapy exposed subjects, as well as to decline in the setting of drug resistance.
- Drug resistance resulting in decreasing viral fitness may explain the immunologic stability seen in subjects with persistent viremia in the setting of multidrug-resistant virus.
- Further research is needed to determine how measures of viral fitness can be used in the clinic.

Expert opinion

Current data strongly suggests that viral virulence and fitness have an impact on the natural history of HIV-1 disease. The best characterized marker of virulence remains the ability of the virus to induce syncytia *in vitro*. Nevertheless, despite extensive research describing the relationship between the emergence of this marker and disease progression, it remains controversial as to whether the emergence of this biologic phenotype is actually the cause or effect of immunologic decline. It is also intriguing to speculate what *in vivo* pressures might select against the evolution of this phenotype. Evidence for this is that while the SI biologic phenotype is determined by relatively few amino acids within the variable V3 loop of the envelope, it tends to occur after may years of infection, and then only in approximately half of HIV-1-infected individuals.

The relationship between the SI phenotype and tropism for CXCR4 allows for the development of assays that utilize molecular methods, as opposed to tissue culture systems for the identification of these strains. While these assays provide valuable insight into HIV-1 pathogenesis, their utility in the clinical management of infected individuals has not been defined. Nevertheless, there is little doubt that understanding how these different viruses exist *in vivo* will be vital as novel antagonists of CCR5 and CXCR4 receptors undergo drug development.

Differences in viral fitness were first hypothesized to exist based upon the unexplained variability in the natural history of HIV-1 disease. Measures of this qualitative virologic marker are complex, particularly since overall fitness must incorporate the intrinsic properties of the virus along with *in vivo* selection pressures. Developing new methods to measure overall viral fitness *in vivo* and *ex vivo* will be important in advancing our understanding of HIV-1 immunopathogenesis. From a clinical perspective, the greatest interest in viral fitness relates to observations that those with high level drug-resistant virus often experience persistent viral suppression along with immunologic stability. The development of standardized, high throughput assays of *pol* RC have allowed for further exploration of the relationship between drug resistance, viral fitness and disease progression. The data presented in this review support the proposition that *pol* RC does indeed predict clinical disease progression in untreated and minimally treated subjects as well as in those with multidrug resistant virus. The next step in the development of this assay will be to demonstrate how it can be utilized in the clinic.

Outlook

The understanding of how HIV-1 virulence and fitness influence the natural history of disease has slowly emerged. The greatest advances in the future may relate to the development of technologies that allow for the real-time evaluation of various markers of virulence and fitness that can be evaluated in large cohorts of carefully characterized individuals. In fact, further development of these assays will likely lead to a better understanding of how these markers can be applied clinically. In the context of biologic phenotype for SI virus and coreceptor tropism, the development of novel entry blockers may lead the way for managing patients in a directed approach based upon the properties of their particular virus. The utility of assays for assessing viral fitness will likely be led by the *pol* RC assay, followed by those that assess the fitness of viruses that develop resistance to novel agents, such as entry and fusion inhibitors. Ultimately, management of those with limited treatment options may well be driven by the use of these measures of fitness, the goal being to use the least number of agents possible to maintain immunologic stability. This strategy will allow for clinicians to minimize the number of drugs used in such patients, reducing cost, toxicities and the potential for the continual evolution of resistant and crossresistant viruses that may limit the utility of drugs in development.

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Affiliation

Eric S Daar, MD
Harbor-UCLA Medical Center,
Division of HIV Medicine,
1124 West Carson St, N-24 Torrance,
CA 90502, USA
Tel.: +1 310 222 2467
Fax: +1 310 533 0447
EDaar@LABioMed.org