The Clinical Implications of Reduced Viral Fitness

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Viral fitness, defined as the extent of viral adaptation to the host environment, arises from tissue tropism, immune system evasion, drug resistance, and viral replication capacity. The fitness of wild-type and drug-resistant HIV-1 varies widely, associating with plasma viremia, CD4+ T-cell count, and clinical progression. HIV-1 fitness may be measured in competitive culture assays, single cycle assays, or single cycle assays based on a subgenomic fragment of HIV-1, which has been standardized as the replication capacity assay (*pol* RC). During virologic failure of antiretroviral therapy, CD4 T-cell counts remain elevated while *pol* RC declines and remains durably lower because of drug-selected changes in the *gag* and *pol* genes. CD4 T-cell sparing also is observed among patients without evidence of drug resistance who carry a low *pol* RC virus. Reduced HIV-1 replication capacity and virulence may occur because of drug resistance or viral escape from host immune responses.

Introduction

Variation in clinical progression

There is wide variation in clinical progression rates and plasma viremia among patients infected with HIV-1 [1]. The basis for variation in disease progression is partly attributable to genetic differences across individuals, such as human leukocyte antigen (HLA) [2]. However, the capacity of HIV-1 to induce disease may vary across viral isolates. There is variation in virologic and immunologic responses to antiretroviral therapy across patients [3], which is mediated by variation in host and viral factors. With the advent of antiretroviral therapy, we have come to appreciate that virologic failure (viral loads not durably suppressed below the lower limits of detection) does not portend clinical failure [4•], suggesting the relationship between high viral replication and clinical virulence may be altered by drug resistance. Multiple lines of evidence indicate the fitness and virulence of HIV-1 vary among treatment-naïve patients and those receiving combination antiretroviral therapy.

Definition: fitness

We define viral fitness as the extent of adaptation by a virus for replication in a defined environment. A virus such as HIV-1, which replicates to high levels in a human host, is highly fit for that environment. The determinants of HIV-1 fitness are complex and include tissue tropism, immune system evasion, drug resistance, and viral replication capacity (Table 1).

Definition: virulence

Virulence is the capacity of the virus to injure the host. HIV-1 is fit and virulent for human hosts because it causes progressive immunodeficiency and death in most infected persons in the absence of effective antiretroviral therapy. HIV-1 is thought to have evolved from a zoonotic transfer of SIVcpz, a primate lentivirus endemic in some chimpanzee colonies, in the first half of the 20th century [5,6]. However, SIVcpz does not cause progressive immunodeficiency [7], and therefore is fit but not virulent for the chimpanzee. Hence, fitness and virulence may be partly or entirely distinct from one another.

Precedent for drug resistance–mediated changes in microbial fitness and virulence

There is precedent in microbiology for resistance-mediated genetic changes to have direct impact on microbial virulence and transmissibility. Drug resistance may be mediated through single amino acid changes, such as the R292K mutant oseltamivir-resistant neuraminidase *Haemophilus influenza* [8], or through horizontal transfer of large blocks of genetic material ("pathogenicity islands") among vancomycin-resistant *Enterococcus faecium* (VRE) [9] or methicillin-resistant *Staphylococcus aureus* (MRSA) [10]. Colonization by VRE may be enhanced over vancomycin-sensitive *E. faecium* but is not usually associated with disease, suggesting determinants of transmission and virulence are distinct in these isolates. In contrast, MRSA is highly transmissible and associated with significant morbidity and mortality [11]. Acyclovir-resistant herpes simplex virus (HSV) 2 is observed among immunocompromised hosts [12], likely because of absence of immune system control, allowing drug pressure to become the dominant selection pressure against the virus. Acyclovir-

Table 1. Determinants of viral fitness

Determinant	Description
Tissue tropism	Ability to enter and replicate in discrete cell populations
Immune system evasion	Ability to evade human immune responses such as neutralizing antibody or cytotoxic T-cell responses
Drug resistance	Ability to replicate in the presence of antiviral compounds
Replication capacity	Ability to replicate in an ideal environment with abundant cellular targets and no inhibitors

resistant HSV-2 is not readily transmitted, which is in marked contrast to the epidemiologically very successful drug-susceptible variants of HSV. Multidrug-resistant *Mycobacterium tuberculosis* (MDRTB) is less likely to appear in case clusters, suggesting these isolates cause fewer secondary cases. However, secondary cases of MDRTB are widely reported, and MDRTB remains pathogenic [13]. Hence, microbiology indicates a spectrum of virulence that is associated with adaptation to drugs or drug resistance. It has not been fully determined where HIV-1 fits in this spectrum.

Measuring the fitness of HIV-1

Viral fitness is described within a defined ecosystem. The ecosystem that exists in humans can be modeled in the laboratory in several ways, each of which has significant limitations.

In vivo fitness

The ideal measure of viral fitness of HIV-1 is observation of in vivo competition of two or more variants by tracking the relative proportion of each virus over time, allowing estimates of relative fitness [14,15]. Assessment of in vivo fitness is difficult because of the need to sample many different tissue sources at frequent time points, followed by labor-intensive, sensitive polymerase chain reaction–based quantitation methods that distinguish between mutant and wild-type variations of the virus. Hence, viral fitness is rarely measured in vivo.

Animal models

The SCID-hu thy/liv mouse model [16] carries a human fetal thymic and kidney tissue explant that recapitulates development of human thymocytes and supports replication of HIV-1.

In vitro fitness assessments

A variety of in vitro systems are used to approximate viral fitness. Distinct viral strains may be introduced into a single in vitro cell culture system. Observation of the rate at which one isolate overgrows one or several other viruses allows estimates of the relative fitness of that virus relative to its competitors. Alternatively, two or more viral isolates may be introduced into separate culture systems under identical conditions and observed for growth rates in the absence of a competing strain. Neither system will recapitulate the selection environment of the host but may provide important information about viral growth kinetics in human cell populations. The lymphoid histoculture system (tonsil) is used to assess fitness and virulence of HIV-1 for lymphocyte populations [17].

In vitro assessment of replication capacity

Replication capacity, the ability of a virus to replicate in an ideal environment, may be assessed through assays that measure the amount of viral replication over a single replication cycle. These assays can use whole viruses derived from culture, provided that replication is limited to a single cycle by limiting the time of the culture or by adding potent inhibitors that block second-round infections. More routinely, single-cycle replication capacity assays are performed using viruses that are genetically manipulated so that they cannot replicate more than one cycle. These assays typically involve inserting part of the viral genome derived from patient-derived tissues into a viral vector that can be used to measure the amount of replication. These assays involve a patient-derived *pol/pro* gene segment, which encompasses viral protease, reverse transcriptase, and several cleavage sites within GAG [18,19].

Clinical Observations Regarding Viral Fitness

Viral load and clinical progression

In 1997, Mellors *et al.* [1] observed that progressively higher levels of plasma HIV-1 RNA were associated with more rapid rates of CD4 decline among patients not receiving antiretroviral therapy. Plasma HIV-1 RNA has been and continues to be a key laboratory predictor of CD4+ T-cell loss [20]. Among treatment-naïve patients with a drug-sensitive virus, antiretroviral therapy is associated with dramatic reductions in plasma viremia, gains in CD4+ T-cell counts, and significant reductions in morbidity and mortality from HIV/AIDS-associated illnesses [21].

Partial virologic responses to combination antiretroviral therapy

In 1995, Eron *et al.* [3] described partial virologic responses to dual therapy of the nucleoside reverse transcriptase inhibitors (NRTI) lamivudine and zidovudine. Regardless of dose, the partial virologic responses of patients receiving combination therapy were more profound than responses of patients receiving monotherapy, and CD4 responses were greater. Resistance to lamivudine is conferred by the M184V mutation and has been associated with partial virologic responses to therapy and lowered viral fitness in vitro [22] and in vivo

[23,24]. The T215Y mutation confers high-level resistance to zidovudine and has been associated with decreased viral fitness [25]. In the absence of treatment, the T215Y mutation does not always revert to the wild-type (T215) and instead may mutate further to T215C, -D, -S, -N, or other variants. These variants are called "shadow" mutations because they indicate prior selection for the resistant mutants. The change from Y to T involves two nucleotide changes, whereas the change from Y to C is only one nucleotide (A to a G in second position). Furthermore, the T215C/D mutants have markedly restored viral fitness, as indicated by their persistence in patients. Shadow mutations appear to place patients at greater risk for emergence or re-emergence of zidovudine resistance [26] because the genetic pathway to resistance is now shortened, and/or the resistant variant may remain at low levels in latently infected cells. This demonstrates how the likelihood of reversion to, or conversion from, a drug-resistant variant is dependent on the complexity of the genetic pathway and the magnitude of the possible fitness gain.

CD4+ expansions during virologic failure of a protease inhibitor

In 1997, Hammer *et al*. [21] reported more profound virologic responses and greater expansion in CD4 T-cell counts among patients receiving indinavir, lamivudine, and zidovudine compared with lamivudine and zidovudine alone. In contrast, Hirsch *et al*. [27] reported comparable CD4+ T-cell gains over 6 months of follow-up among patients receiving indinavir monotherapy and patients receiving a combination of indinavir, zidovudine, and lamivudine, although virologic responses were better with the three-drug regimen. It was speculated that protease inhibitors (PIs) might spare CD4 cell counts through direct inhibition of cellular proteasomes critical for antigen presentation by major histocompatibility complex [28]. Alternatively, drug resistance mutations within protease might exact a viral fitness cost. For example, viral protease D30N and L90M mutations selected by PIs have been associated with decreased viral fitness [29]. Mammano *et al*. [19,30] and Zennou *et al*. [31] performed some of the earliest, detailed phenotypic and functional characterizations of PI-resistant HIV-1. Mutations to PIs have been associated with reductions in viral enzymatic efficiency [32], inappropriate cleavage of *gag* proteins [31], and changes in peptide specificity [33].

CD4 preservation during virologic drug failure: has virulence been lowered?

Ledergerber *et al*. [4•] observed that patients receiving a PI-based regimen who had achieved an initial decline in viral load to the limit of detection but subsequently experienced rebound had an equivalent risk for clinical progression compared with patients who maintained complete suppression. In contrast, patients who never achieved undetectable levels had an increased risk for progression, possibly reflecting low drug exposure because of poor adherence. In a separate study, patients in long-term virologic failure of a PI-based regimen [34] were compared to untreated patients from the San Francisco Men's Health Study [35]. The patients in virologic drug failure demonstrated significantly slowed CD4 T-cell loss and even sustained increases in CD4 T-cell counts at levels of viremia associated with rapid CD4 loss in untreated patients [34]. These findings suggested the in vivo virulence of HIV-1 was decreased among patients in long-term virologic failure of a PI-based regimen. Among patients unable to suppress plasma viral load to below the limits of detection, the degree of change in plasma viremia from pretherapy levels proved to be a better predictor of CD4 responses than the absolute level of viremia [34].

Recovery of viral fitness in a patient with resistance to ritonavir monotherapy

Nijhuis *et al*. [36] performed a detailed study of one patient administered ritonavir monotherapy to determine if continued viral evolution under drug pressure would restore fitness lost with the development of PI resistance. The patient's virus was derived at time points from pretherapy baseline to day 115. The protease gene was cloned into a reference virus (HXB2) and assayed in cell culture for viral growth compared to a recombinant virus bearing the patient's baseline wild-type protease. The authors also assessed the catalytic efficiency of the patient's viral protease for its cognate *p6* cleavage site in GAG. A viral isolate of nearly wild-type replication efficiency emerged by day 28, followed by the emergence on day 82 and 115 isolates that each exceeded wild-type. After an initial 200 CD4+ cell per μL gain, by day 55 of monotherapy CD4 counts had resumed decline. Resistant variants, which emerged in the first 60 days of therapy, replicated poorly compared to wild type and did not show detectable protease enzymatic activity. However, the day 82 isolate bore a protease of greater catalytic efficiency (k_{cat}/K_m) than the wild-type protease. This work suggested that PI-resistant viruses isolated from patients in long-term virologic failure of a PI-based regimen might experience rapid viral fitness recovery and force a resumption of CD4+ T-cell loss.

Replication capacity not recovered by protease inhibitor–resistant patients receiving combination therapy

These careful observations of one patient failing PI monotherapy do not address the likelihood of fitness recovery in viruses that are adapting to a PI and NRTI during combination therapy. Subsequent clinical observation demonstrated that patients in long-term virologic failure of a PI-based regimen including at least two NRTIs sustained partial viral load suppression and elevated CD4 counts for more than 3 years [37].

We observed 20 patients who had previously experienced virologic failure of antiretroviral therapy, from time of salvage on a new PI-based regimen [38•]. Each patient rapidly developed high-level phenotypic resistance to the prescribed PI and additional resistance to NRTIs. As resistance increased rapidly, replication capacity (*pol* RC) was observed to decrease to low levels and in proportion to the level of initial decline in plasma viremia. *pol* RC remained low over time, despite viral evolution toward greater phenotypic resistance to PIs and NRTIs, and increasing plasma viremia levels. Continued viral evolution did not act to restore *pol* RC. Primary resistance mutations were observed to generally lower viral *pol* RC, whereas secondary mutations had a sum neutral effect and might lower or raise *pol* RC. Contrary to observations by Nijhuis *et al.* [36], the appearance of secondary resistance mutations was not generally "compensatory" for *pol* RC. The durability of CD4 protection appeared linked to *pol* RC durably reduced to below 45% of control virus. Change in viral load from pretherapy levels proved to be a better predictor of CD4 change compared to absolute viral load because it tracks changes in viral replication capacity. In this way, partial virologic responses among patients with drug resistance are a measure of the degree of reduction in the fitness and virulence of HIV-1. *pol* RC may not have recovered because of complex mutation patterns and coevolution between protease and the *gag* cleavage sites (*eg*, the rate limiting *p6* cleavage site) [39] contained in the viral construct.

Replication capacity and virulence restored after stopping a protease inhibitor–based regimen

In a study of 16 patients in long-term (average 30 months) virologic failure of a PI-based regimen, viral replication capacity was observed to be decreased compared to a distribution of wild-type viruses [40•]. When patients were randomized to stop therapy, patients undergoing treatment cessation experienced outgrowth of an archived, wild-type virus of increased viral replication capacity. Viral loads increased shortly after cessation of therapy but before the emergence of wild-type virus. The early increase in viral load before any appearance of better-replicating, drug-susceptible virus indicates that the partial viral load suppression observed during therapy is caused, in part, by continued antiviral activity against the partially resistant virus. The timing of the emergence of the wild-type virus coincided with a second abrupt increase in viral load to baseline levels and resumption of rapid loss of CD4 cells (Fig. 1). A highly virulent form of HIV-1 returned when the low *pol* RC, drug-resistant virus was overgrown. Hence, partial virologic responses during virologic drug failure are attributable to continued antiviral activity against partially resistant viruses and drug selection that maintains poorly replicating viruses.

Biological Basis for CD4-Sparing Clinical Phenotype

In vitro assessments of fitness and virulence of protease inhibitor–resistant HIV-1

Liegler *et al.* [41] assayed PI-resistant isolates drawn from patients with partial virologic suppression and sustained CD4 counts. Although somewhat delayed, the PI-resistant viruses eventually achieved similar viral loads and remained cytopathic for CD4 cells in a highly activated, phytohemagglutinin-stimulated, peripheral blood mononuclear culture system. In this system, tropism was a strong determinant of target cell depletion, whereas drug resistance was not. Penn *et al.* [42] introduced PI-resistant isolates into a complex tissue culture system of human tonsil tissue. In this model system of human lymphoid tissue, the PI-resistant viruses replicated to high levels, indicating a high capacity for replication, and displayed equal virulence for CD4 T cells. The results of Liegler *et al.* [41] and Penn *et al.* [42] suggested that the replication capacity deficit of PI-resistant isolates may be overcome in lymphoid cell populations with a high cellular activation state, which effectively rescues viruses with low replication capacity that retain wild-type levels of virulence regarding their capacity to deplete target cells. In each of these models, the drug-resistant variants depleted target cells in proportion to the amount of virus replication, indicating that the specific virulence (the amount of cell death per unit of replication) of drug-resistant HIV-1 in these systems is preserved. These model systems are consistent with clinical observations in which partial CD4 T-cell responses are correlated with partial viral load responses.

Drug resistance and tropism

Stoddart *et al.* [43] demonstrated that PI-resistant virus clones did not replicate in human thymic explants and in SCID-hu thy/liv mice, resulting in complete preservation of thymocytes, relative to infections by a drug-sensitive homologue. The drug-resistant viruses also replicated slightly less well on activated lymphocytes [41], but the replication impairment was much more severe in thymic tissues. Reduced viral fitness in tissues critical to CD4 development, expansion, and/or storage, such as thymus or resting lymphocytes, may be the basis for CD4+ T-cell count preservation observed in patients bearing PI-resistant isolates. These results highlight that viral fitness varies across compartments, virulence is determined in part by tropism for the target tissue, and that immune activation may be an important factor that affects the amount of viral replication and target cell depletion.

How low *pol* replication capacity virus preserves CD4 counts

Low *pol* RC HIV-1 may not replicate well in thymus or other tissues, allowing maturation of thymocytes into naïve CD4+ T cells, replenishing depleted memory and effector CD4+ populations in the periphery. PI-resistant,

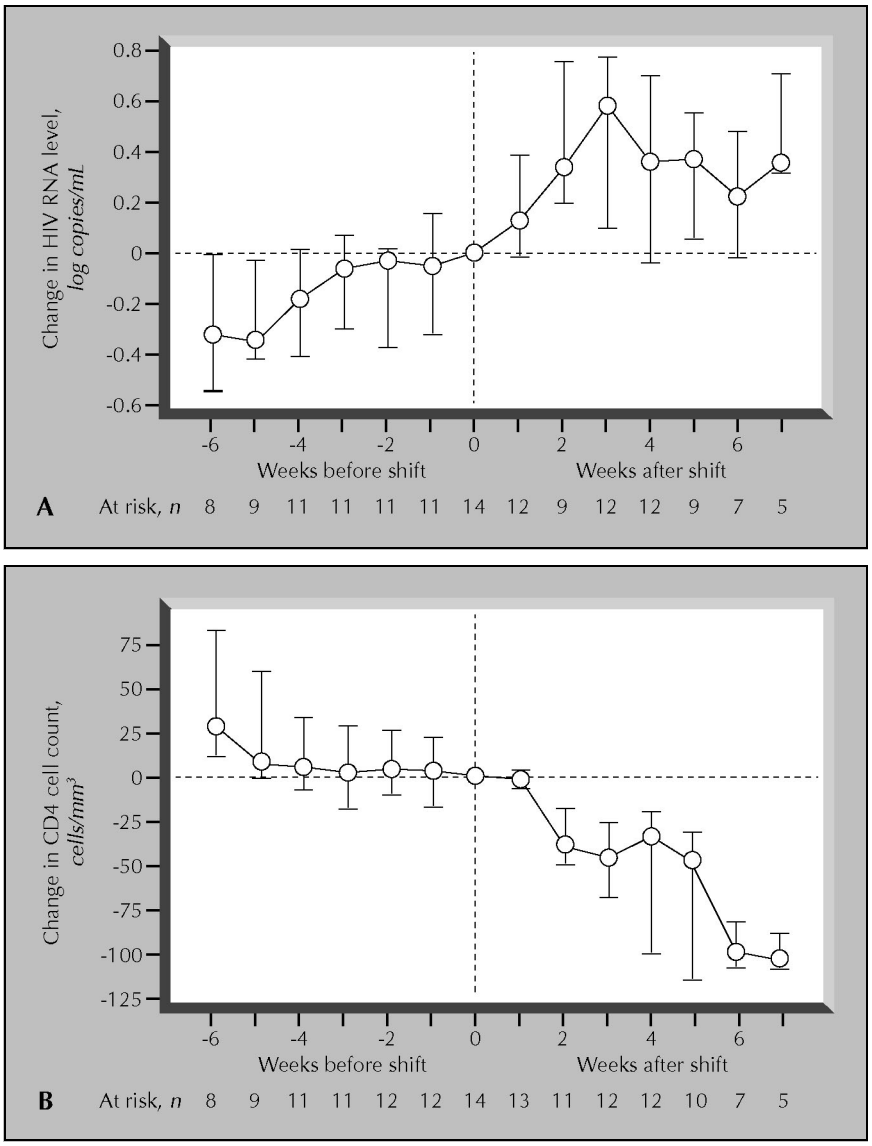

Figure 1. **A** and **B**, 16 patients in long-term virologic failure of a protease inhibitor–based regimen elected to stop antiretroviral therapy. After a median 6-week period, drug-sensitive HIV-1 of restored replication capacity emerged. Coincident with the emergence of drug-sensitive HIV-1 was a dramatic increase in HIV-1 RNA levels and a resumption of accelerated CD4+ T-cell loss. These results indicate a virulent form of HIV-1 returned when drug resistance mutations were lost and replication capacity was restored [40•]. Reprinted with permission from *The New England Journal of Medicine* (copyright 2001, Massachusetts Medical Society; all rights reserved).

low *pol* RC viruses may be less likely to induce high-level cellular activation [37] associated with CD4+ T-cell loss [44]. Alternatively, low *pol* RC may be a consequence of robust immune responses that force genetic changes in the virus that lower *pol* RC. Patients with robust immune responses against the virus may not experience dysregulation into the high activation states associated with CD4+ T-cell loss in HIV-1 disease.

Wild-type fitness variation

There is substantial variation in the fitness of wild-type, drug-sensitive isolates of HIV-1. Quinones-Mateu *et al.* [45] used a dual competition assay to demonstrate that variants from long-term nonprogressors were outcompeted by variants from patients with progressive disease. Viral *pol* RC has been observed to vary widely among recently infected patients without phenotypic and genotypic evidence of drug resistance [46]. Among patients with *pol* RC lowered to below 43%, we observed significant preservation of CD4 counts (663 versus 512 cells/μL, $P = 0.004$). CD4 counts remained elevated over time in the absence of treatment. After initiation of treatment, patients with low *pol* RC maintained higher CD4 counts and showed evidence of greater increases in CD4 counts after 1 year of treatment, despite having comparable levels of virologic response to therapy. The threshold *pol* RC value of 43% observed among treatment-naïve patients with a drug-sensitive virus was similar to that observed in patients in long-term virologic failure of a PI-based regimen with sustained CD4 counts [47]. Daar *et al.* [48] recently reported delayed disease progression rates at lowered levels of *pol* RC in a cohort of HIV-infected hemophiliacs with progressive disease.

Immune escape and viral fitness changes during the course of infection

The genetic basis of lowered *pol* RC among wild-type, drug-sensitive viruses is just now being investigated, but the first

identified genetic correlates may by under HLA pressure [49]. Moore *et al*. [50] have demonstrated HLA-restricted patterns of viral evolution in the viral reverse transcriptase gene of adults infected with HIV-1. The authors detected polymorphisms at sites included in known HLA-restricted epitopes and identify other sites likely to be under control by specific HLA alleles. Position 135 of reverse transcriptase was much more likely to change away from wild-type isoleucine to another residue (odds ratio = 93, $P < 0.0001$) among patients carrying a B*5101 allele, which is known to present an epitope containing the 135 position. Barbour *et al*. [49] have independently observed that change from the wild-type isoleucine at position 135 of reverse transcriptase is associated with lowered viral replication capacity. Taken together, these results suggest that immune escape from HLA class I pressure may be more likely to occur at residue changes that lead to lowering of viral fitness. Modulation of the fitness and virulence of HIV-1 by the human immune system is an important future area of research.

Resistance and viral fitness changes to non-nucleoside reverse transcriptase inhibitors and new drug classes

Clinical resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) may increase, decrease, or have no effect on viral fitness. Little *et al*. [51] have suggested that patients in early infection with an NNRTI-resistant virus have higher plasma HIV-1 RNA levels. Huang *et al*. [52] observed that NNRTI mutations V106A, G190C/S, P225H, M230L, and P236L lowered *pol* RC. Archer *et al*. [53] and Gerondelis *et al*. [54] reported that K103N, V106A, and Y181C mutations, each of which are commonly observed in patients with resistance to NNRTIs, do not significantly impact fitness in vitro. It is unsurprising that the effect of NNRTI resistance on fitness should vary, given the position of the NNRTI-binding site. All NNRTIs bind a common pocket distant from the reverse transcriptase–active site. Hence, NNRTI mutations are likely to exert their effects through interactions that cascade through the protein to the active site. These effects on viral enzyme efficiency may be buffered or enhanced in certain genetic backgrounds. In contrast, primary resistance mutations to PIs and reverse transcriptase generally fall close to the active site of the enzyme. The impact of resistance to new drug classes such as integrase inhibitors or fusion inhibitors (inhibitors of viral envelope contact with host cell surface proteins) on the in vivo virulence and fitness of HIV-1 is unclear and will need to be carefully monitored over time. Early reports suggest resistance mutations to diketo acid–based inhibitors of integrase are associated with decreased enzyme catalysis and viral replication in vitro [55].

Conclusions

Decreased *pol* RC has been associated with partial suppression of viremia and preservation of CD4 counts over time. PI-resistant, multidrug-resistant HIV-1 has lowered *pol* RC and is less virulent [38•,40•], with CD4 counts remaining elevated significantly above pretreatment levels for more than 3 years [37]. We have observed development of resistance and steadily increasing viremia, but not recovery of replication capacity or virulence among patients with PI-resistant, multidrug-resistant HIV-1 receiving continuous treatment with combination antiretroviral therapy [38•]. The effect of multidrug resistance on *pol* RC, associated partial virologic response, and CD4 cell sparing appears to be durable in some patients followed over 2 to 3 years. Additional follow-up is needed to determine if viruses that are drug-resistant and fully virulent will evolve. Experience indicates that evolution of full virulence and resistance can occur for some but not all microbes. Tracking HIV-1 evolution with respect to resistance and virulence is an area of active investigation.

The standardized *pol* RC assay has been shown to correlate well with estimates of in vivo fitness after therapy is stopped [15,40•]. Nonetheless, it remains surprising that a viral construct including only 10% of the HIV-1 genome should correlate with in vivo changes in viral load and CD4 T-cell counts. The viral protease and reverse transcriptase measured in the *pol* RC assay are two of only three enzymes (the other is integrase) encoded by HIV-1, and play crucial roles in the lifecycle of HIV. Protease cleavage of the *gag* polyprotein mediates virion assembly, maturation, and therefore, viral infectivity. Reverse transcriptase mediates transcription of the viral RNA into viral DNA for insertion into the host DNA through viral integrase. Reductions in the activity of protease or reverse transcriptase may have profound impact on viral replication. The cleavage sites within *gag*, and the individual structural proteins coded within, are known to genetically vary inside and outside of the context of antiretroviral therapy and may significantly impact viral fitness. Modification of the *pol* RC assay to include all of GAG may enhance the predictive power and potential utility of the assay.

It is unknown if drug-resistant HIV-1 is less likely to be transmitted. The prevalence of drug-resistant forms among newly infected patients may be lower than the proportion of exposures to drug-resistant forms of HIV-1 [56]. Moreover, few of the recently infected patients with drug resistance carry a true high-level multidrug-resistant variant, which is often associated with lowered viral fitness [51,57]. Drug resistance may lower risk for transmission because of partial virologic suppression. Risk for transmission is greater at higher viral loads; hence, any reduction in viremia, even if only a partial reduction, should reduce risk [58].

The clinical utility of replication capacity assays has not been defined. The assays appear to have some prognostic value for predicting subsequent trends in CD4 counts in treated and untreated patients, but additional information is needed to evaluate whether baseline replication capacity assessment provides additional predictive value after

considering baseline levels of CD4 T-cell counts, viral load, T-cell activation, and viral tropism. The prognostic value of replication capacity measurements remains to be confirmed with additional, larger studies. Clinically relevant cut-offs for predicting subsequent virologic and immunologic events will need to be defined. Once this occurs, research can be planned to evaluate the role of replication capacity measurements in clinical decision making, including how often to monitor untreated patients, when to start antiretroviral therapy, when to switch therapy after virologic failure, and what intensity of therapy to prescribe (*ie*, how many drugs). Such clinical evaluation of laboratory tools requires several years, as it has for viral load and drug resistance assays. In the meantime, these measurements are providing insights into the basic relationships between viral evolution and virulence, which underlie all aspects of the HIV/AIDS epidemic.

Extending life, reducing morbidity, and restoring immune competence are the central goals of antiretroviral therapy in HIV-1 disease. This is best accomplished by complete suppression of viral replication. For patients with extensively cross-resistant viruses who cannot achieve improvements in virologic response using new agents, continued treatment to maintain a virus of lowered *pol* RC is superior to cessation of treatment. For maximum clinical benefit and for public health prevention measures to halt the growth of the epidemic, every reasonable attempt should be made to reduce and maintain viral replication to the lowest levels possible.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Mellors JW, Munoz A, Giorgi JV, *et al.*: **Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection.** *Ann Intern Med* 1997, **126:**946–954.
2. Carrington M, O'Brien SJ: **The influence of HLA genotype on AIDS.** *Annu Rev Med* 2003, **54:**535–551.
3. Eron JJ, Benoit SL, Jemsek J, *et al.*: **Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party.** *N Engl J Med* 1995, **333:**1662–1669.
4. • Ledergerber B, Egger M, Opravil M, *et al.*: **Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study.** *Lancet* 1999, **353:**863–868.

This was the first report indicating that virologic failure of combination antiretroviral therapy was not associated with increased rates of clinical progression.

5. Korber B, Muldoon M, Theiler J, *et al.*: **Timing the ancestor of the HIV-1 pandemic strains.** *Science* 2000, **288:**1789–1796.
6. Hahn BH, Shaw GM, De Cock KM, Sharp PM: **AIDS as a zoonosis: scientific and public health implications.** *Science* 2000, **287:**607–614.
7. Peeters M, Fransen K, Delaporte E, *et al.*: **Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee.** *AIDS* 1992, **6:**447–451.
8. Carr J, Ives J, Kelly L, *et al.*: **Influenza virus carrying neuraminidase with reduced sensitivity to oseltamivir carboxylate has altered properties in vitro and is compromised for infectivity and replicative ability in vivo.** *Antiviral Res* 2002, **54:**79–88.
9. Shankar N, Baghdayan AS, Gilmore MS: **Modulation of virulence within a pathogenicity island in vancomycin-resistant Enterococcus faecalis.** *Nature* 2002, **417:**746–750.
10. Baba T, Takeuchi F, Kuroda M, *et al.*: **Genome and virulence determinants of high virulence community-acquired MRSA.** *Lancet* 2002, **359:**1819–1827.
11. Enserink M: **Resistant staph finds new niches.** *Science* 2003, **299:**1639–1641.
12. Erlich KS, Mills J, Chatis P, *et al.*: **Acyclovir-resistant herpes simplex virus infections in patients with the acquired immunodeficiency syndrome.** *N Engl J Med* 1989, **320:**293–296.
13. Barnes PF, Cave MD: **Molecular epidemiology of tuberculosis.** *N Engl J Med* 2003, **349:**1149–1156.
14. Bonhoeffer S, Barbour AD, De Boer RJ: **Procedures for reliable estimation of viral fitness from time-series data.** *Proc R Soc Lond B Biol Sci* 2002, **269:**1887–1893.
15. Grant RM, Liegler T, Elkin C, *et al.*: **Protease inhibitor resistant HIV-1 has marked decreased fitness in vivo.** *Paper presented at the 8th Conference on Retroviruses and Opportunistic Infections.* Chicago, IL, February 4–8, 2001.
16. Namikawa R, Kaneshima H, Lieberman M, *et al.*: **Infection of the SCID-hu mouse by HIV-1.** *Science* 1988, **242:**1684–1686.
17. Glushakova S, Baibakov B, Zimmerberg J, Margolis LB: **Experimental HIV infection of human lymphoid tissue: correlation of CD4+ T cell depletion and virus syncytium-inducing/non-syncytium-inducing phenotype in histocultures inoculated with laboratory strains and patient isolates of HIV type 1.** *AIDS Res Hum Retroviruses* 1997, **13:**461–471.
18. Petropoulos CJ, Parkin NT, Limoli KL, *et al.*: **A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1.** *Antimicrob Agents Chemother* 2000, **44:**920–928.
19. Mammano F, Petit C, Clavel F: **Resistance-associated loss of viral fitness in human immunodeficiency virus type 1: phenotypic analysis of protease and gag coevolution in protease inhibitor-treated patients.** *J Virol* 1998, **72:**7632–7637.
20. Yeni PG, Hammer SM, Carpenter CC, *et al.*: **Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel.** *JAMA* 2002, **288:**222–235.
21. Hammer SM, Squires KE, Hughes MD, *et al.*: **A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team.** *N Engl J Med* 1997, **337:**725–733.
22. Back NK, Nijhuis M, Keulen W, *et al.*: **Reduced replication of 3TC-resistant HIV-1 variants in primary cells due to a processivity defect of the reverse transcriptase enzyme.** *Embo J* 1996, **15:**4040–4049.
23. Frost SD, Nijhuis M, Schuurman R, *et al.*: **Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection.** *J Virol* 2000, **74:**6262–6268.
24. Marschner IC, Collier AC, Coombs RW, *et al.*: **Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy.** *J Infect Dis* 1998, **177:**40–47.
25. Goudsmit J, de Ronde A, de Rooij E, de Boer R: **Broad spectrum of in vivo fitness of human immunodeficiency virus type 1 subpopulations differing at reverse transcriptase codons 41 and 215.** *J Virol* 1997, **71:**4479–4484.
26. Riva C, Violin M, Cozzi-Lepri A, *et al.*: **Transmitted virus with substitutions at position 215 and risk of virological failure in antiretroviral-naive patients starting highly active antiretroviral therapy.** *Paper presented at the XI International HIV Drug Resistance Workshop.* Sevilla, Spain, July 2–5, 2002.

27. Hirsch M, Steigbigel R, Staszewski S, *et al.*: **A randomized, controlled trial of indinavir, zidovudine, and lamivudine in adults with advanced human immunodeficiency virus type 1 infection and prior antiretroviral therapy.** *J Infect Dis* 1999, **180:**659–665.
28. Andre P, Groettrup M, Klenerman P, *et al.*: **An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T cell responses.** *Proc Natl Acad Sci USA* 1998, **95:**13120–13124.
29. Martinez-Picado J, Morales-Lopetegi K, Wrin T, *et al.*: **Selection of drug-resistant HIV-1 mutants in response to repeated structured treatment interruptions.** *AIDS* 2002, **16:**895–899.
30. Mammano F, Trouplin V, Zennou V, Clavel F: **Retracing the evolutionary pathways of human immunodeficiency virus type 1 resistance to protease inhibitors: virus fitness in the absence and in the presence of drug.** *J Virol* 2000, **74:**8524–8531.
31. Zennou V, Mammano F, Paulous S, *et al.*: **Loss of viral fitness associated with multiple Gag and Gag-Pol processing defects in human immunodeficiency virus type 1 variants selected for resistance to protease inhibitors in vivo.** *J Virol* 1998, **72:**3300–3306.
32. Croteau G, Doyon L, Thibeault D, *et al.*: **Impaired fitness of human immunodeficiency virus type 1 variants with high-level resistance to protease inhibitors.** *J Virol* 1997, **71:**1089–1096.
33. Dauber DS, Ziermann R, Parkin N, *et al.*: **Altered substrate specificity of drug-resistant human immunodeficiency virus type 1 protease.** *J Virol* 2002, **76:**1359–1368.
34. Deeks SG, Barbour JD, Martin JN, *et al.*: **Sustained CD4+ T cell response after virologic failure of protease inhibitor-based regimens in patients with human immunodeficiency virus infection.** *J Infect Dis* 2000, **181:**946–953.
35. Winkelstein W Jr, Samuel M, Padian NS, Wiley JA: **Selected sexual practices of San Francisco heterosexual men and risk of infection by the human immunodeficiency virus.** *JAMA* 1987, **257:**1470–1471.
36. Nijhuis M, Schuurman R, de Jong D, *et al.*: **Increased fitness of drug resistant HIV-1 protease as a result of acquisition of compensatory mutations during suboptimal therapy.** *AIDS* 1999, **13:**2349–2359.
37. Deeks SG, Hoh R, Grant RM, *et al.*: **CD4+ T cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy.** *J Infect Dis* 2002, **185:**315–323.
38.• Barbour JD, Wrin T, Grant RM, *et al.*: **Evolution of phenotypic drug susceptibility and viral replication capacity during long-term virologic failure of protease inhibitor therapy in human immunodeficiency virus-infected adults.** *J Virol* 2002, **76:**11104–11112.

This paper demonstrated that continued viral evolution toward high phenotypic resistance to PI and NRTI components of antiretroviral therapy did not lead to recovery of replication capacity. Secondary resistance mutations, which were observed in most patients, did not "compensate" and restore *pol* RC, thereby allowing CD4 counts to remain elevated for the duration of observation.

39. Gatanaga H, Suzuki Y, Tsang H, *et al.*: **Amino acid substitutions in Gag protein at non-cleavage sites are indispensable for the development of a high multitude of HIV-1 resistance against protease inhibitors.** *J Biol Chem* 2002, **277:**5952–5961.
40.• Deeks SG, Wrin T, Liegler T, *et al.*: **Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia.** *N Engl J Med* 2001, **344:**472–480.

This paper demonstrated that cessation of therapy among patients in long-term virologic failure of a PI-based regimen was associated with return of a drug-sensitive, highly virulent form of HIV-1 with restored replication capacity. The study demonstrated that there is a residual antiviral effect of the failing regimen against the resistant viruses, indicating that drug resistance is usually partial. Partial viral load responses were correlated with changes in replication capacity.

41. Liegler TJ, Hayden MS, Lee KH, *et al.*: **Protease inhibitor-resistant HIV-1 from patients with preserved CD4 cell counts is cytopathic in activated CD4 T lymphocytes.** *AIDS* 2001, **15:**179–184.
42. Penn ML, Myers M, Eckstein DA, *et al.*: **Primary and recombinant HIV type 1 strains resistant to protease inhibitors are pathogenic in mature human lymphoid tissues.** *AIDS Res Hum Retroviruses* 2001, **17:**517–523.
43. Stoddart CA, Liegler TJ, Mammano F, *et al.*: **Impaired replication of protease inhibitor-resistant HIV-1 in human thymus.** *Nat Med* 2001, **7:**712–718.
44. Hazenberg MD, Otto SA, van Benthem BH, *et al.*: **Persistent immune activation in HIV-1 infection is associated with progression to AIDS.** *AIDS* 2003, **17:**1881–1888.
45. Quinones-Mateu ME, Ball SC, Marozsan AJ, *et al.*: **A dual infection/competition assay shows a correlation between ex vivo human immunodeficiency virus type 1 fitness and disease progression.** *J Virol* 2000, **74:**9222–9233.
46. Grant RM, Barbour JD, Wrin T, *et al.*: **Transmission of drug resistant HIV-1 exhibiting lower replication capacity is associated with higher CD4 cell counts.** *Paper presented at the XI International HIV Drug Resistance Workshop.* Sevilla, Spain, July 2–5, 2002.
47. Barbour JD, Hecht FM, Wrin T, *et al.*: **Higher CD4+ T cell counts associated with low viral pro/pol replication capacity among treatment-naïve adults in early HIV-1 infection.** *J Infect Dis* 2004, In press.
48. Daar ES, Kesle K, Lail A, *et al.*: **HIV co-receptor tropism and replication capacity predict HIV progression [abstract H-1722c].** *Paper presented at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy.* Chicago, IL, September 14–17, 2003.
49. Barbour JD, Wrin T, Deeks SG, *et al.*: **Examination of wide variation in replication capacity of wild-type HIV-1: analysis of genotype-phenotype association via tree-structured methods.** *Paper presented at the XII International HIV Drug Resistance Workshop.* Los Cabos, Mexico, June 10–14, 2003.
50. Moore CB, John M, James IR, *et al.*: **Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level.** *Science* 2002, **296:**1439–1443.
51. Little SJ, Holte S, Routy JP, *et al.*: **Antiretroviral-drug resistance among patients recently infected with HIV.** *N Engl J Med* 2002, **347:**385–394.
52. Huang W, Gamarnik A, Limoli K, *et al.*: **Amino acid substitutions at position 190 of human immunodeficiency virus type 1 reverse transcriptase increase susceptibility to delavirdine and impair virus replication.** *J Virol* 2003, **77:**1512–1523.
53. Archer RH, Dykes C, Gerondelis P, *et al.*: **Mutants of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase resistant to nonnucleoside reverse transcriptase inhibitors demonstrate altered rates of RNase H cleavage that correlate with HIV-1 replication fitness in cell culture.** *J Virol* 2000, **74:**8390–8401.
54. Gerondelis P, Archer RH, Palaniappan C, *et al.*: **The P236L delavirdine-resistant human immunodeficiency virus type 1 mutant is replication defective and demonstrates alterations in both RNA 5'-end- and DNA 3'-end-directed RNase H activities.** *J Virol* 1999, **73:**5803–5813.
55. Hazuda DJ, Felock P, Witmer M, *et al.*: **Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells.** *Science* 2000, **287:**646–650.
56. Leigh Brown AJ, Frost SD, Mathews WC, *et al.*: **Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population.** *J Infect Dis* 2003, **187:**683–686.
57. Grant RM, Hecht FM, Warmerdam M, *et al.*: **Time trends in primary HIV-1 drug resistance among recently infected persons.** *JAMA* 2002, **288:**181–188.
58. Gray RH, Wawer MJ, Brookmeyer R, *et al.*: **Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda.** *Lancet* 2001, **357:**1149–1153.