Novel Viral Markers Predict HIV Disease Progression

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Over the past 20-plus years of the AIDS epidemic, clinicians have come to appreciate that while there are relatively predictable markers of HIV disease progression—viral load and CD4+ cell counts being the most widely utilized—the course of HIV infection is extremely variable among individuals. "Everyone is familiar with the graph showing peak in viremia and then the virologic set point that's achieved, followed by a progressive decline in CD4+ cells and the development of symptoms, usually in about eight to ten years after the point of infection," Dr. Eric Daar explained. "But there's a lot of heterogeneity among individuals in this regard. There are some individuals who have high viral set points, with their CD4+ cells declining more quickly, some even progressing to AIDS within the first 12 to 24 months. At the other end of the spectrum are the unusual long-term nonprogressors, who maintain viral suppression, high CD4+ cell counts, and remain completely asymptomatic for many years. And in between, we see varying rates of disease progression."

Building upon these observations, researchers have been determined to unlock the mysteries of the elusive factors—and biological markers—associated with HIV disease progression. "If we could better understand why some people are long-term nonprogressors, we may be able to apply this learning as a therapeutic for those with progressive disease," Dr. Daar added. "Short of this, we also have much to learn from patients with varying rates of HIV disease progression, especially the host and virologic factors involved. Additional tools are needed to help clinicians better predict their patients' risk of clinical progression."

I. Determinants of Disease Progression: Host Factors

Over the past ten to 15 years, a number of determinants have been identified as being factors associated with disease progression (or lack thereof). Some of the more recent research has focused on host factors, including mutations within chemokine receptors and HLA types.

Mutations within Coreceptors

In 1996, two HIV coreceptors—CXCR4 (originally called fusin) and CCR5—were identified. It was determined that CCR5 was the receptor responsible for the fusion of macrophage-tropic (M-tropic) HIV, whereas the CXCR4 chemokine coreceptor was found to be used by T-cell tropic primary isolates of HIV (See "Coreceptor Tropism" below). While X4 viruses are rarely transmitted, they evolve in about half of HIV-infected people. When they do, they are associated with a more rapid disease course.

With these discoveries, researchers were able to determine that the CD4+ cells of some individuals who appeared to be immune to HIV infection contained a 32-base pair deletion in both of their genes coding for the CCR5 coreceptor. These mutations are seen in only a small percentage of people of European descent; about 15% have mutations in one of the genes (dubbed a heterozygous deletion), and between 1% and 2% have mutations in both of them (dubbed a homozygous deletion). These genetic mutations are even more rarely detectable in people of either Asian or African ancestry.

Individuals with a heterozygous deletion have been shown to benefit from slower disease progression (Smith, 1997; de Roda Husman, 1997). This protection seems to result from a combination of reduced expression of CCR5 on cells and increased production of the chemokines (RANTES, MIP-1α and MIP-1β) that are the natural binding agents (or "ligands") for this receptor. This means that HIV has fewer CCR5 receptors for docking onto cells and less of a chance to find CCR5 receptors that are not already being used by these binding proteins Similar chemokine receptor defects include polymorphisms in the CCR2 receptor and the stroma-derived factor type-1 (SDF) among others (Hogan, 2001).

HLA Alleles

The major histocompatibility complex (MHC) class I, better known as human leukocyte antigen (HLA) class I in humans, play a pivotal role in the response of cytotoxic T-lymphocytes (CTLs) to HIV-infected CD4+ cells in the body. A large number of HLA alternative forms (HLA alleles) exist in the human population, some of which can increase the risk and progression of certain human diseases, and each individual expresses six major class I alleles. One of the strongest links is between HLA-B27 and the autoimmune disease ankylosing spondylitis; individuals who possess HLA-B27 are approximately 90 times more likely to develop the disease than other individuals in the population.

HLA alleles also appear to contribute to the progression of HIV infection. Studies have documented that HLA-B27, HLA-B51, and HLA-B57 are strongly associated with slower rates of HIV disease progression, whereas a certain subtype of the HLA-B35 and HLA-A29 alleles among others are associated with significantly faster rates of disease progression.

HLA-B57 is a noteworthy specific example. In one recent study, the relationship between HLA-B57 and clinical presentation in acute HIV infection in two large acute HIV infection cohorts established in Boston and San Francisco was assessed (Altfeld, 2003). A total of 59 individuals from San Francisco and 57 individuals from Boston had presented with acute symptomatic HIV infection and were typed for HLA class I alleles at the time of the study. Only four of these 116 individuals (3.4%) expressed the HLA class I allele B57. The low prevalence of HLA-B57 in individuals with symptomatic acute HIV infection was significantly different from the percentage of individuals expressing this allele in the general HIV-infected population (9.6%), as determined by HLA class I typing of 446 infected individuals from the Boston area. These findings suggest that symptomatic acute infection is less frequent in persons who are HLA-B57 positive.

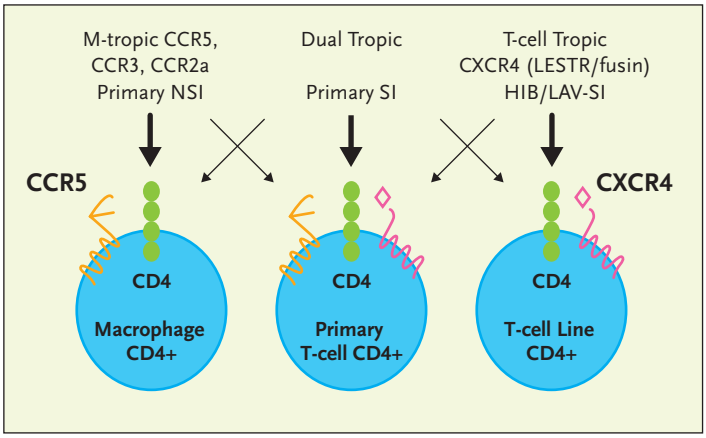

FIGURE 1. **HIV Cell Tropism**

The evaluation of the coreceptor tropism of HIV may provide highly useful information at all stages of HIV clinical care. Viruses isolated shortly after infection tend to infect macrophages, rather than T cells, and do not cause cells to fuse into a multinucleated syncytium. Such viruses are said to be M-tropic strains. Later in the course of infection, viral isolates infect T-cells and do induce the formation of syncytia. The emergence of these viruses, known as T-tropic strains, is often accompanied by a decline in the CD4+ cell count and, if left unchecked, the development of AIDS. The different tropisms are predominantly determined by the sequential interaction of the viral envelope (env) glycoproteins with CD4 and one of two coreceptors. Syncytium-inducing (SI) HIV strains primarily use the CXCR4 coreceptor on CD4+ cells (referred to as X4 strains), whereas non-syncytium-inducing (NSI) strains use the CCR5 coreceptor (referred to as R5 strains). Also pictured here are dual-tropic virus that can use both CCR5 and CXCR4.

Source: William A. O'Brien, MD, MS

It was also determined that, during acute HIV infection, HIV-specific T-cell responses were dominated by HLA-B57-restricted responses, with significantly broader and stronger responses restricted by HLA-B57 than restricted by all other co-expressed HLA class I alleles combined. Six out of nine individuals expressing HLA-B57 controlled HIV viremia—less than 5,000 HIV-RNA copies/mL—in the absence of therapy for up to 29 months following acute infection.

II. Determinants of Disease Progression: Immunologic Factors

CELLULAR IMMUNE RESPONSES—SPECIFICALLY HIV-SPECIFIC CELLULAR immune responses—are believed to play a pivotal role in the control of HIV replication. Perhaps some of the best known work focusing on cellular immune responses has been conducted by Drs. Bruce Walker, Eric Rosenberg, Marcus Altfeld, and Marylyn Addo, all situated at the Partners AIDS Research Center in Boston and all past PRN lecturers.

As has been reviewed in numerous articles published in past editions of *The PRN Notebook*, individuals who maintain low levels of viremia—in the absence of antiretroviral therapy—have been shown to have robust and consistent HIV-specific cytotoxic T-lymphocyte (CTL) responses to the virus. However, robust HIV-specific immune responses have also been seen in patients with progressive disease. But in these patients, the presence of HIV-specific CTL responses has not generally been associated with viral control. This observation led to the hypothesis, which is currently being worked out in studies being conducted at the Partners AIDS Research Center and elsewhere, that control of HIV infection may have more to do with the *quality* of virus-specific responses directed at HIV than the actual *quantity* of HIV-specific CTLs present.

For a more extensive review of the immunologic factors believed to be associated with slower disease progression, please see the article "HIV Long-Term Nonprogression: Insights into Pathogenesis and Viral Control" in the December 2003 issue of the *Notebook*.

III. Determinants of Disease Progression: Virologic Factors

MUCH OF DR. DAAR'S TALK FOCUSED ON VIROLOGIC FACTORS BELIEVED TO be associated with varying rates of HIV disease progression, particularly coreceptor tropism and viral fitness.

Coreceptor Tropism

VIRUSES ISOLATED SHORTLY AFTER AN INDIVIDUAL BECOMES POSITIVE for HIV tend to infect macrophages, as well as T cells, and do not cause certain cell lines to fuse into a multinucleated syncytium. Such viruses are referred to as M-tropic strains (see Figure 1). Later in the course of infection, viral isolates infect T-cells, but not macrophages, and do induce the formation of syncytia. The emergence of these viruses, known as T-tropic strains, is often accompanied by a decline in the CD4+ cell count and, if left unchecked, the development of AIDS.

The different tropisms are predominantly determined by the sequential interaction of the viral envelope (env) glycoproteins with CD4 and one of two coreceptors. Syncytium-inducing (SI) HIV strains primarily use the CXCR4 coreceptor on CD4+ cells (referred to as X4 strains), whereas non-syncytium-inducing (NSI) strains use the CCR5 coreceptor (referred to as R5 strains). "There are lots of studies trying to better understand what's going on with coreceptors tropism," Dr. Daar explained. "The long and short of it is that there's clearly an association between the stage of disease and the presence of SI or X4 viruses, even though many people progress to AIDS without the emergence of these strains."

Knowing a patient's SI/NSI phenotype has predictive value and there are a number of ongoing longitudinal studies looking at this. In one study reported in 1995, a group headed by Dr. Daar analyzed viral load and SI/NSI phenotype in samples of sequentially cryopreserved peripheral blood mononuclear cells (PBMCs) collected from eight HIV-positive patients participating in the Multicenter AIDS Cohort Study (MACS) (Daar, 1995). Three patients remained clinically and immunologically stable over a five- to eight-year period, three demonstrated precipitous declines in their CD4+ cell counts, and two had gradual declines in their CD4+ cell counts.

Viral load remained relatively low in those who remained clinically and immunologically stable, while increasing substantially in all five individuals who experienced a decline in CD4+ cells. Two patients were noted to have a switch from NSI to SI isolates immediately preceding a precipitous decline in CD4+ counts, while the third individual who experienced such a decline—along with the two patients who experienced gradual CD4+ cell count declines—did not develop SI iso-

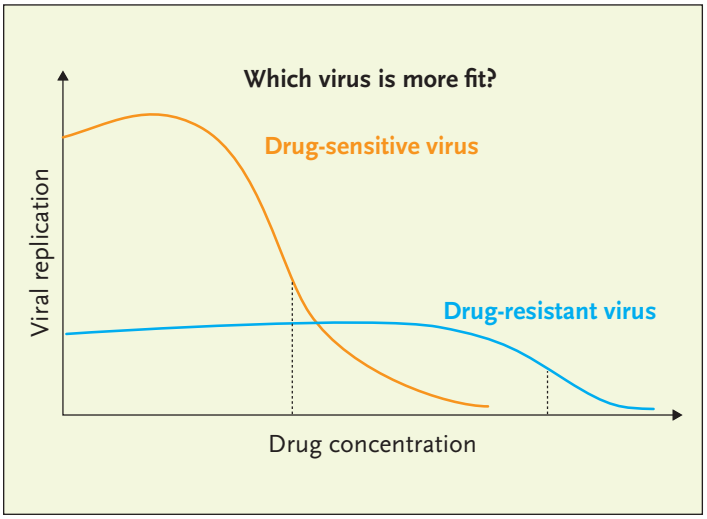

FIGURE 2. **Replication Capacity and Viral Fitness**

Viral fitness is a complex evolutionary term used to describe HIV's replicative adaptability in a given environment, typically in competition with other strains under similar conditions. A true measure of viral fitness would be one that analyzes the ability of a patient's entire virus to grow in the *in vivo* milieu, influenced by immunologic and drug pressures. However, such measures are not readily available. What is available is an *ex vivo* measure of relative viral replication as defined by variable virologic factors. When HIV becomes resistant to antiretroviral drugs, it often does so at a cost to the virus' replication capacity. Research has suggested that a measurement of replication capacity can provide valuable information that, when used in combination with drug-resistance results, can facilitate treatment decisions.

Source: ViroLogic, Inc.

lates. While the study was too small to draw any firm conclusions, it did show that HIV behavior in clinically and immunologically stable patients was characterized by relatively low viral replication and suggested a predominance of NSI isolates.

"There are many other studies just like this one, where patients with stable CD4+ cell counts always appear to have NSI virus," Dr. Daar added. "Upon identifying patients who have a more precipitous decline in CD4+ cells, what we frequently find is an associated switch from NSI to SI, right around the time in which the CD4+ cells plummet."

In another study, conducted by Drs. Douglas Richman and Sam Bozzette, both of the University of California, San Diego, samples collected from 325 patients enrolled in 11 antiretroviral therapy clinical trials were evaluated for SI virus and correlated with both CD4+ cell declines and clinical endpoints (Richman, 1994). Adjusted mean rates of CD4+ count decline were 40 and 102 cells/mm^3 per year in the NSI and SI groups respectively. Rates of CD4+ count decline in 16 patients who converted from NSI to SI virus averaged 31 cells/mm^3 per year before conversion, compared to 142 cells/mm^3 per year afterward. And in a nested case-control analysis, patients who experienced immunologic or clinical decline were 2.3 to 3.5 times more likely to have SI virus than were controls. Based on these results, Drs. Richman and Bozzette concluded that the presence of the SI virus is believed to be a strong predictor of CD4+ cell decline and progression of disease. However, they pointed out that when controlling for the CD4+ cell count, SI virus did not prove to increase the immediate risk of death.

More recently, Dr. Daar's group presented data at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) showing in a cohort of longitudinally followed HIV-infected hemophiliacs that the presence of any X4 virus at baseline was associated with a marked increased risk of clinical progression to AIDS compared to those without such strains (Daar, 2003). These data, Dr. Daar explained, show that the relationship between coreceptor tropism and disease progression is similar to what was previously seen with biologic phenotype analyses.

Nevertheless, Dr. Daar pointed out that it's extremely difficult, at this point in time, to confirm a cause and effect relationship. There has been no shortage of *in vitro* data suggesting that R5 viruses are less cytopathic than X4 strains. In turn, it is widely believed that it is the switch in coreceptor tropism and associated biologic phenotype that leads to the decline in the CD4+ cell count. "The reality is, we can't really say this," Dr. Daar commented. "It's possible that it may be immunologic collapse that allows for the emergence of SI viruses. Only a few mutations with the hypervariable portion of HIV, the V3 loop, are necessary for a switch from R5 to X4 virus. One intriguing possibility is that there may be some selective pressure against the emergence of these highly virulent viruses. Otherwise, you'd anticipate that they would emerge in virtually everybody. But they clearly don't."

"The conclusion with respect to coreceptor tropism is that it clearly is associated with differences in the natural history of disease," Dr. Daar explained. "Clearly, it's time to examine the potential role of X4 blocking agents. But I think what's really needed are more pathogenesis-based studies to better understand the factors associated with the emergence of X4 viruses. Whether X4 virus is causing CD4+ cell decline or immunologic decline leads to the emergence of X4 virus, we need to understand what's causing it to happen and to figure out what is selecting against it."

Viral Fitness

VIRAL FITNESS IS A COMPLEX EVOLUTIONARY TERM USED TO DESCRIBE HIV'S replicative adaptability in a given environment, typically in competition with other strains under similar conditions (see Figure 2). A true measure of viral fitness would be one that analyzes the ability of a patient's entire virus to grow in the *in vivo* milieu, influenced by immunologic and drug pressures. However, such measures are not readily available.

What is available is an *ex vivo* measure of relative viral replication as defined by variable virologic factors, in the form of ViroLogic's replication capacity (RC) assay, using the PhenoSense phenotypic testing technology as its platform. The assay is currently used on all samples submitted for phenotypic testing (RC of a patient's HIV is included in the lab report sent to clinicians by ViroLogic).

The ViroLogic RC assay is a multi-step process (see Figure 3 on page 6). First, recombinant viruses for RC testing are generated by introducing resistance test-vector DNA into cell cultures (transfection), along with an expression vector that includes a mouse retrovirus envelope protein. After transfection, virus is harvested from cells and the amount of virus produced is evaluated. The virus is then inoculated into new cell cultures and further incubated. After normalization for the amount of input virus, luciferase activity detected in the infected cells is determined and used as a direct measure of "infectivity," (i.e., the ability of the virus to infect target cells [dependent on portions of gag, protease, and reverse transcriptase]). The relative RC is assessed by comparing the amount of luciferase activity produced by sample-derived viruses to the amount of luciferase activity produced by a well-characterized population of wild-type isolates from HIV-infected individuals. RC measurements are finally expressed as a percent of the wild-type reference virus (e.g., 70%).

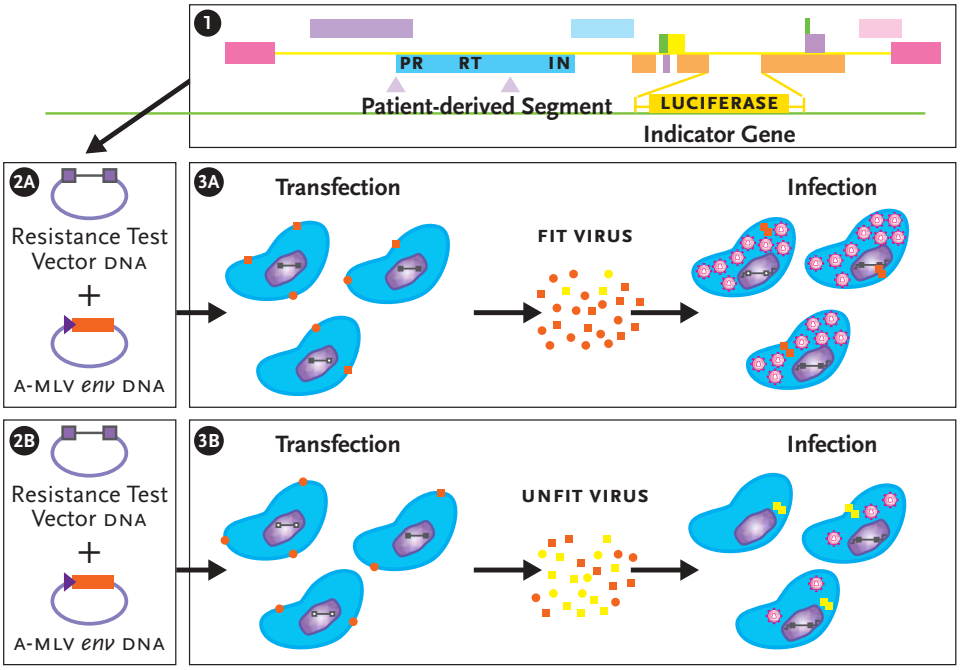

FIGURE 3. **Replication Capacity Laboratory Process**

The replication capacity assay represents a modification of ViroLogic's PhenoSense HIV phenotypic drug susceptibility assay. The assay begins by assembling resistance test vectors (RTV) (1). RTVs contain protease and reverse transcriptase sequences derived from the patient virus and a luciferase gene to measure virus replication. Cells are cotransfected with RTV-DNA and a murine leukemia virus (MLV) envelope expression vector to generate MLV env pseudotyped virus particles, which are used to infect target cells (2a and 2b). Replication capacity is measured by comparing the amount of luciferase activity produced in the patient virus to that of a reference virus (typically the NL4-3 strain). If the virus replicates well, it will produce large amounts of luciferase in infected cells (3a). It the virus has impaired replication capacity it will produce reduced amounts of luciferase in infected cells (3b). Because the assay only requires a single round of virus replication, it can be completed in eight to 10 days.

Source: ViroLogic, Inc.

"The ViroLogic assay only assesses the impact that selected portions of the virus have on viral fitness," Dr. Daar commented. "Plus, it is only measuring fitness in the *in vitro* setting, separate from the influence of the immune response, target cell availability, drug pressure, and other selective forces encountered *in vivo*. If we are to use this test in the clinic or to explore HIV pathogenesis, we need to know that it is a reliable and reproducible measure. We also need to determine its clinical relevance. Finally, we need to define if, in fact, we should be using it."

The Clinical Significance of RC and Drug-Resistant HIV

FOR MOST CLINICIANS CARING FOR HIV-POSITIVE PATIENTS, INTEREST IN viral fitness and RC has been greatest in the context of immunologic discordance: the maintenance or continual increase in CD4+ cell counts and clinical improvement in the face of virologic failure while on antiretroviral therapy. "We all remember, back in 1996, when we started patients on protease inhibitor-based regimens and they all did really well," recalled Dr. Daar. "However, we also saw patients who didn't do so well virologically and we also saw patients who initially responded well to therapy who subsequently experienced rebounds in viral load. Most of the time, these observations were the result of emerging drug resistance. But the thing is, many of these patients were still doing well clinically and immunologically."

In one early analysis involving the Swiss HIV Cohort Study, 98 HIV-positive patients with extensive prior antiretroviral therapy began a protease inhibitor-based regimen in 1997 (Kaufmann, 1998). A year later, 66% of the patients were unable to achieve or maintain viral loads below 500 copies/mL. However, when comparing patients who were and were not able to maintain undetectable viral load, there was no statistically significant difference in the CD4+ cell response rates. In patients with persistently detectable viremia, there was an average CD4+ count increase of 105 cells/mm^3 after 48 weeks. And once therapy was discontinued, the CD4+ cell counts typically dropped to baseline levels. "A hypothesis to explain this observation was that the predominant virus population in the setting of drug pressure is the fittest virus, but this drug-resistant virus is relatively unfit compared to wild-type virus," Dr. Daar explained. "When you take away drug pressure, the more fit wild-type virus emerges as the predominant virus, which is associated with CD4+ cell decline."

To test this hypothesis, Dr. Steven Deeks of the University of California, San Francisco (UCSF) and his colleagues evaluated the virologic and immunologic consequences of discontinuing antiretroviral therapy in HIV-infected patients with detectable viremia (Deeks, 2001). The primary objective of this study was to determine whether antiretroviral therapy provides continued benefit—despite large reductions in drug susceptibility—and to identify the virologic mechanisms responsible for any continued benefit. A component of this objective was to measure the RC of the patients' drug-resistant HIV, using the ViroLogic assay described above.

The study had two components. The first was a nonblinded, prospective study of 16 patients who were randomly assigned to either discontinue (n=11) or continue (n=5) antiretroviral therapy. The second was a nonrandomized, prospective, observational study in which seven patients discontinued therapy.

Drug resistance remained stable during the 12 weeks of observation in the five patients who continued antiretroviral therapy. In contrast, protease inhibitor susceptibility shifted to wild-type levels within 16 weeks in 9/10 (90%) patients in the randomized component of the study who discontinued therapy (the other patient in this group had no detectable resistance to protease inhibitors at study entry and was not included in this analysis). As for patients who discontinued therapy in the nonrandomized component of the study, susceptibility to protease inhibitors shifted to wild-type levels in 6/7 (86%).

The shift in drug susceptibility was often abrupt, occurred at various times after therapy was discontinued, and usually occurred for all drugs simultaneously. Among all 15 patients who discontinued therapy and had a shift in drug susceptibility, the median time from the discontinuation of therapy to the initial waning of protease inhibitor resistance was six weeks. Once resistance began to wane, it disappeared completely after a median of two weeks.

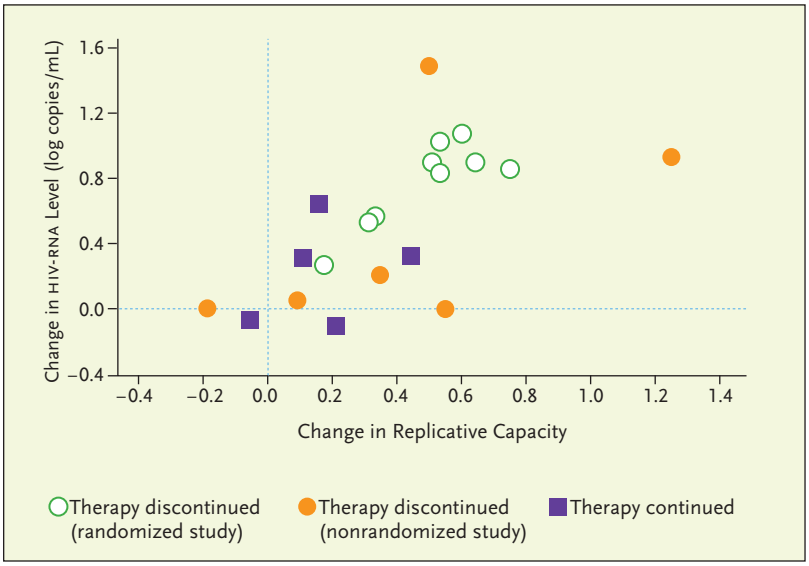

FIGURE 4. Change in Viral Load Compared with the Change in RC Between Baseline and Week 12

In this study, Dr. Steven Deeks and his colleagues hypothesized that the emergence of wild-type virus during a structured treatment interruption might be associated with increased viral replication. Analyzed were the changes in viral load during the time drug resistance began to wane. Twelve of the 14 patients who discontinued therapy and had an abrupt shift in protease inhibitor susceptibility were included in the analysis. Replicative capacity was calculated as the ratio of the luciferase activity from vectors containing patient-derived sequences to the luciferase activity from vectors containing reference sequences. Relative to a wild-type reference virus, the median RC of vectors derived from 22 viral samples obtained at baseline was 20%. In most samples, the RC increased between baseline and week 12. This increase was greater in the patients who were randomly assigned to discontinue therapy than in those assigned to continue therapy and there was a significant correlation between the change in plasma HIV-RNA levels and the change in RC during the 12 weeks of evaluation.

Source: Deeks, 2001.

Dr. Deeks and his group hypothesized that the emergence of wild-type virus might be associated with increased viral replication. To test this, they analyzed the changes in viral load during the time drug resistance began to wane. All 14 patients who discontinued therapy and had an abrupt shift in protease inhibitor susceptibility were included in the analysis (three patients excluded from the analysis because one had no detectable protease inhibitor resistance at study entry, one had no change in protease inhibitor resistance, and one had only a gradual loss of protease inhibitor resistance). Viral loads and CD4+ cell counts were stable immediately before the onset of the shift to a drug-susceptible virus and changed rapidly as the level of drug resistance waned. After the shift to wild-type virus, viral loads were significantly higher than those measured when the shift began. Similarly, CD4+ cell counts decreased significantly after the shift to drug-susceptible virus.

Relative to a wild-type reference virus, the median RC of vectors derived from 22 viral samples obtained at baseline was 20%. In most samples, the RC increased between baseline and week 12. This increase was greater in the patients who were randomly assigned to discontinue therapy than in those assigned to continue therapy and there was a significant correlation between the change in plasma HIV-RNA levels and the change in RC during the 12 weeks of evaluation (see Figure 4).

Also of interest are data presented by Dr. Michael Miller of Gilead Sciences and his colleagues at the 10th Conference on Retroviruses and Opportunistic Infections, held in Boston in 2003 (Miller, 2003). This study evaluated the impact of a range of mutations selected by NRTIs on replication capacity *in vitro*, the results of which are shown in Figure 5 on page 8.

The Clinical Significance of RC in Untreated HIV

JUST AS VIRAL LOAD IS VARIABLE AND IS ASSOCIATED WITH CLINical progression in patients who have not yet initiated antiretroviral therapy, there is reason to believe that RC is also variable in treatment-naive patients and, as a result, may have a predictive value. This was the suggestion stemming from the conclusion of one study conducted by investigators at the Gladstone Institute of Virology and Immunology of UCSF, published in the July 15, 2004 issue of the *Journal of Infectious Diseases* (Barbour, 2004).

In this study, blood samples were collected from 191 patients enrolled in the San Francisco Options Project, a cohort of acutely and newly infected HIV-positive individuals. Patients were referred to the Options Project from a variety of sources, including physicians and HIV testing sites and by self-referral. All individuals were excluded from the cross-sectional analysis if they had received antiretroviral therapy prior to enrollment.

The presence of drug-resistant variants was assessed using Bayer Diagnostic's Trugene genotypic assay and RC was assessed using ViroLogic's phenotypic-based assay.

The median CD4+ count at baseline was 519 cells/mm^3 and the median viral load was 4.75 $\log_{10}$ copies/mL. Drug resistance–associated mutations were identified in 35 (18.6%) viruses at study entry: seven (3.9%) had primary protease inhibitor (PI) mutations, 22 (11.2%) had nucleoside reverse transcriptase inhibitor (NRTI) mutations, and 18 (10.3%) had non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations.

The median RC at study entry was 69% of the standard control. There was no difference in RC by approximate length of infection, as assessed by the three different study-entry eligibility categories. RC was significantly lower among patients infected with a PI-resistant virus than for those infected with a virus without evidence of drug resistance. Patients infected with a virus with NRTI and/or NNRTI-resistant virus tended to have lower RC, although they did not significantly differ from those infected with a virus without genotypic evidence of drug resistance. Similarly, while lower RC was associated with a greater number of primary PI-resistance mutations, it was not associated with a greater number of NRTI- or NNRTI-resistance mutations.

At study entry, RC was not significantly associated with viral load, but was significantly—and negatively—associated with CD4+ cell counts. In other words, patients with the highest CD4+ cell counts tended to be harboring virus with the lowest RC. Patients infected with a virus with an RC of <42% had an average of 663 CD4+ cells/mm^3 at study entry, compared to patients infected with a virus replicating at >42% capacity, who had 512 CD4+ cells/mm^3.

Of the 191 patients studied at study entry, 65 patients were followed for an average of 447 days before initiating antiretroviral therapy. Patients infected with a virus with an RC value of >42% at study entry had lower CD4+ cell counts over time (137.3 fewer CD4+ cells/mm^3). Adjusting for viral load did not alter the effect of RC on CD4+ cell counts over time. What's more, the inferences and significance trends were not al-

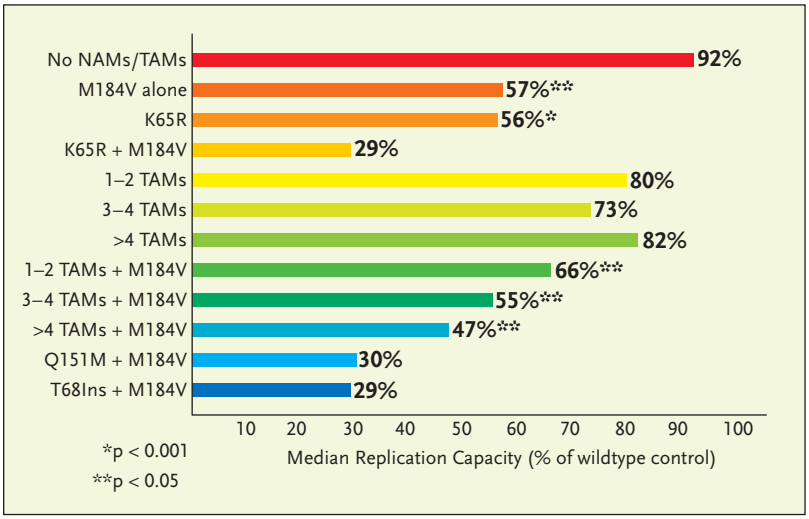

FIGURE 5. Replication Capacity of HIV with Various NRTI-Associated Resistance Mutations

In a study presented at the 10th Conference on Retroviruses and Opportunistic Infections, the replication capacity of various viral isolates with a variety of NRTI resistance mutations was analyzed. Analyzed was the replication capacity of 1040 HIV isolates that had NRTI but no protease inhibitor resistance mutations. The mutations commonly associated with failure of a tenofovir (Viread)-inclusive and lamivudine (Epivir)-inclusive regimen—M184V and K65R—led to a median replication capacity of 57% and 56% respectively. The median replication capacity of isolates harboring both the M184V and K65R mutations was 29%. Also shown here are the median replication capacity results involving isolates with various thymidine analogue mutations (TAMs), alone and in combination with M184V.

Source: Miller, 2003.

tered when patients infected with a drug-resistant virus were excluded from the analysis.

Also of interest are data evaluating RC and CD4+ cell counts in patients who initiated antiretroviral therapy. Of the 191 patients, 122 (64%) elected to receive treatment, after consultation with their clinicians. Of these, 114 were followed for a median of 33 days. Among patients with an RC value of >42% at study entry, CD4+ cell counts remained significantly lower than among those infected with a virus with a low RC. Moreover, poorer virologic responses to antiretroviral therapy were independently associated with lower CD4+ cell counts.

Returning to his group's data reported at the 43rd ICAAC, Dr. Daar further discussed the relationship between baseline RC and the natural history of disease in a cohort of hemophiliacs (Daar, 2003). It is important to note that these subjects had received either no antiretroviral therapy or were on monotherapy at the time the assay was performed. Dr. Daar's group demonstrated that there was a significant correlation between RC and baseline CD4+ cell counts and that increasing RC was significantly associated with an increased risk of CD4+ cell count decline, even after controlling for baseline CD4+ cell counts, HIV-RNA levels, and coreceptor tropism. They further showed that increasing RC predicted an increased risk of progression to clinical AIDS, even if controlling for the CD4+ cell count and viral load. Together, with the recent data from the Gladstone Institute, these data strongly support that RC is a virologic marker associated with the natural history of HIV disease.

Conclusion

UNDER WHAT CIRCUMSTANCES MIGHT CLINICIANS THINK about employing RC? "Once could argue that it has predictive value," Dr. Daar reiterated. "Maybe we would use it to predict progression. However, I'm not sure that it's going to be a terribly useful tool in this setting." He explained that RC is much more likely to be of use in managing patients with limited treatment options. "If we could better understand how we might manipulate the virus to alter the history of disease, when undetectable viral loads are not possible, then we could potentially make a huge impact by allowing us to use focused therapy in these people, while at the same time limiting the amount, cost, and toxicities of therapy."

While this does sound like an appealing option, Dr. Daar made it clear that a lot more work—and a lot more data—is needed to better understand how RC should be used in the clinical setting. However, Dr. Daar commented that, "I do think we are close enough to understanding that RC is a reliable and clinically relevant measure and that we can now design clinical trials to answer this important question." prn

References

Altfeld M, Addo MM, Rosenberg ES, et al. **Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection.** *AIDS* 17(18):2581-91, 2003.

Barbour JD, Hecht FM, Wrin T, et al. **Higher CD4+ T cell counts associated with low viral pol replication capacity among treatment-naive adults in early HIV-1 infection.** *J Infect Dis* 190:251-6, 2004.

Daar ES, Kesler K, Lail A, et al. **HIV coreceptor tropism (CRT) and replication capacity (RC) predict HIV progression** [Abstract H-1722c]. 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 2003.

Daar ES, Chernyavskiy T, Zhao JQ, et al. **Sequential determination of viral load and phenotype in human immunodeficiency virus type 1 infection.** *AIDS Res Hum Retroviruses* 11(1):3-9, 1995.

de Roda Husman AM, Koot M, Cornelissen M, et al. **Association between CCR5 genotype and the clinical course of HIV-1 infection.** *Ann Intern Med* 127:882-90, 1997.

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* 344:472-480, 2001.

Hogan CM, Hammer SM. **Host determinants in HIV infection and disease. Part 2: genetic factors and implications for antiretroviral therapeutics.** *Ann Intern Med* 134(10):978-96, 2001.

Kaufmann D, Pantaleo G, Sudre P, et al. **CD4-cell count in HIV-1-infected individuals remaining viraemic with highly active antiretroviral therapy (HAART): Swiss HIV Cohort Study.** *Lancet* 351(9104):723-4, 1998.

Miller MD, White KL, Petropoulos CJ, et al. **Decreased replication capacity of HIV-1 clinical isolates containing K65R or M184V RT mutations** [Abstract 616]. 10th Conference on Retroviruses and Opportunistic Infections, Boston, 2003.

Richman DD, Bozzette SA. **The impact of the syncytium-inducing phenotype of human immunodeficiency virus on disease progression.** *J Infect Dis* 169(5):968-74, 1994.

Smith MW, Dean M, Carrington M, et al. **Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression.** Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* 277:959-65, 1997.