

HIV Long-Term Nonprogression: Insights Into Pathogenesis and Viral Control

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WHEN IT COMES TO THE NATURAL HISTORY OF HIV INFECTION, VERY FEW generalizations can be made. After an individual is infected with the virus, progression to AIDS—provided that antiretroviral therapy is not used—typically occurs within ten years. “This basically depends on the steady-state viral load,” explained Dr. Marylyn Addo. “Patients who maintain a high viral load after infection has been established are likely to develop AIDS faster than those with lower viral loads.” However, for a small group of individuals, HIV infection runs an unusually benign course. These patients, dubbed long-term nonprogressors (LTNPs), maintain very low viral loads and stable CD4+ cell counts for many years—indeed in some cases—without the assistance of antiretroviral therapy. And it is these LTNPs that have been the intense focus of several research teams around the world. Understanding the factors that contribute to long-term nonprogression will hopefully yield greater insights into the pathogenesis of HIV infection and will be crucial for vaccine design and the development of therapeutic modalities.

Host and viral factors associated with long-term nonprogression have been identified and explored over the past decade. Much of Dr. Marylyn Addo's work—representative of an even larger field of research being conducted at the Partners AIDS Research Center in Boston—has focused on the T-cell responses directed against HIV being seen in LTNPs. This article reviews some of the more recent work under way, based on a June 2003 lecture delivered by Dr. Addo.

Viral Factors and Long-Term Nonprogression: Attenuated Virus

THE TRANSMISSION OF FAULTY VIRUS—ATTENUATED HIV—HAS BEEN SAID to be a factor associated with long-term nonprogression (see Figure 1). In an initial report, dating back to 1992, Jennifer Learmont and her colleagues with the New South Wales Red Cross Blood Transfusion Service in Sydney, Australia, were conducting follow-up on individuals infected with HIV through blood transfusions (Learmont, 1992). The group identified eight subjects who had been infected through a single common donor, all of whom appeared to be experiencing significantly slower rates of HIV disease progression. Seven to more than 10 years after being infected with the virus, the donor and the eight recipients remained free of clinical symptoms and were maintaining normal CD4+ cell counts, compared to more obvious signs of disease progression in individuals who had been infected by other HIV-positive donors around the same time. An analysis of viral isolates collected from these individuals confirmed that they had been infected with a strain of HIV-1 (subtype B) with a conserved deletion of 150 or more base pairs (bp) in the Nef-LTR overlap region and with duplications and rearrangements of nuclear factor- κ B (NF- κ B) and Sp1 transcription factor binding sites in the LTR—a significantly less fit, attenuated form of HIV. [EDITOR'S NOTE: *Follow-up data from this cohort has documented HIV disease progression, warranting initiating of antiretroviral therapy, in the original donor and several of the recipients.*]

Another interesting report was published in 2002 by a team of in-

vestigators at Yale University School of Medicine (Alexander, 2002). The team studied a 15-year-old HIV-positive girl infected at birth who had maintained consistently low HIV-RNA levels as well as normal and stable CD4+ cell counts. She had not presented with any clinical manifestations of AIDS, despite having only received zidovudine (Retrovir) monotherapy for a part of her life. Her HIV-positive mother had also not progressed to AIDS and had never been treated with antiretroviral agents. Viral sequences taken from the 15-year-old girl were found to contain a two-amino-acid insertion mutation in the Vif gene. Vif sequences harbored by her mother contained the identical mutation.

The Vif sequences harbored by both individuals replicated in PBMCs to levels approximately 20-fold lower than that of wild-type virus. Removal of the insertion mutation from this recombinant restored replication efficiency to wild-type levels, while introduction of the insertion mutation into wild-type Vif sequences resulted in greatly decreased replication. Furthermore, Vif protein from the girl's HIV was aberrantly cleaved, suggesting a mechanism for loss of Vif function.

In a more recent study reported by investigators at the Ottawa Health Research Institute in Ontario, a high frequency of R77Q mutations in viral protein R (Vpr)—which induces apoptosis and may contribute to HIV-induced CD4+ cell depletion—was documented in LTNPs, but not in patients with progressive disease (Lum, 2003).

Host Factors and Long-Term Nonprogression: 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, the most common primary strain of the virus transmitted from one person to another. The CXCR4 chemokine coreceptor was found to be used by T-cell tropic primary isolates of HIV, also known as syncytium-inducing (SI) viruses. While SI viruses are rarely transmitted, they evolve in about half of HIV-infected people within five years of infection. 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 the genes for the CCR5 receptor. 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 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-1 α and MIP-1 β) that are the natural binding agents (or “ligands”) for this receptor. This means that HIV has fewer CCR5 receptors

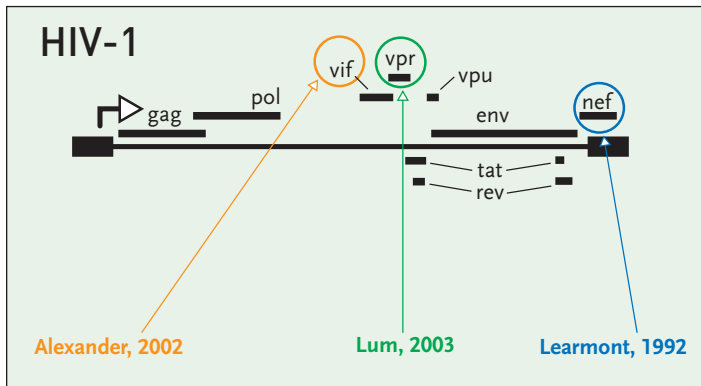


Figure 1. Viral Factors and Long-Term Nonprogression: Attenuated Virus

The transmission of attenuated HIV is believed to be a factor associated with long-term nonprogression. Depicted here are the three HIV genome regions that have been documented in studies to contain mutations associated with long-term nonprogression (citations listed in references).

Source: Marylyn Addo, MD, PhD

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 changes in the CCR2 receptor and the stroma-derived factor type-1 (SDF) among others (Hogan, 2001).

Host Factors and Long-Term Nonprogression: HLA Alleles

THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC), BETTER KNOWN AS human leukocyte antigen (HLA) 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. 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 and HLA-B57 and others 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 much faster rates of disease progression (O'Brien, 2001).

“What has been learned from the study of HLA alleles, chemokine receptor mutations, and other genetic determinants, has shown us that a person’s genetic makeup can play a significant role in determining how well he or she will do in HIV disease,” Dr. Addo commented.

Host Factors and Long-Term Nonprogression: Cellular Immune Responses

MUCH OF THE WORK BEING CONDUCTED AT PARTNERS AIDS RESEARCH Center has focused on “HIV controllers,” which essentially involve two distinct groups of patients: traditional LTNPs, who have maintained low viral loads and high CD4+ cell counts on their own accord over a prolonged period of time (13.5 years, on average), and those who have been identified in the primary stages of HIV infection and have managed to

maintain low or undetectable viremia levels in the absence of antiretroviral therapy (discussed in several past Notebook articles reviewing lectures by Drs. Eric Rosenberg, Marcus Altfeld, and Bruce Walker). “We prefer the term ‘HIV controllers’ to LTNPs because some of our patients who have been able to maintain very low viral loads and normal CD4+ cell counts in the absence of treatment haven’t necessarily been infected for many years,” Dr. Addo explained. “Maybe in 20 years we can categorize them as LTNPs. Right now, however, it’s a bit premature to call all of them LTNPs.”

As explained by Dr. Addo, cellular immune responses—specifically HIV-specific cellular immune responses—are believed to play a pivotal role in the control of HIV replication. In turn, HIV-specific T-cells have remained a primary focus of Dr. Addo’s work.

Upon entering the body via mucosal surfaces, dendritic cells transport HIV to lymphoid tissue-draining reproductive organs and oral and intestinal mucosal surfaces. From there, HIV is widely disseminated to other lymphoid organs, where it is passed on to cells migrating through the lymphoid tissues, its primary targets being CD4+ cells. Once viral RNA has been converted to proviral DNA and integrated into the CD4+ cell’s nucleus, new viral proteins are produced and assembled. At the same time, even before virions are formed, some viral proteins are chopped up, a process known as proteolytic degradation or antigen processing. These proteins are then combined with an HLA class I molecule on the surface of the cell, which in turn signals the immune system, alerting it to the presence of a foreign invader. “It’s basically the cell saying ‘hello, I’m infected, please kill me’,” explained Dr. Addo.

Once the immune system has been primed, cellular responses are generated in response to HIV infection. Cytotoxic T-lymphocytes (CTLs) can kill infected cells—either by direct contact (cytolytic response) or by suppressing HIV replication through the release of soluble antiviral factors (noncytolytic response). However, this is possible only if the CTLs are present in the proper activation state and in sufficient number.

HIV is like many viral infections—including CMV, EBV, and HSV—in that it is not associated with eradication from the human host. Unlike HIV, however, these other viral infections are generally associated with long-term control by the immune system. In rodent models, the immune response to lymphocytic choriomeningitis virus (LCMV) is analogous to the immune response to these viral infections in humans: LCMV-specific CTLs are produced and maintained, ultimately keeping viremia in check.

In HIV controllers, investigators at Partners AIDS Research Center have repeatedly demonstrated the presence of robust and consistent HIV-specific CTL responses. 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 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.

Epitope Analysis of HIV-Specific Responses

AS DISCUSSED BY DR. ADDO, RECENT ADVANCES IN LABORATORY METHODOLOGY, particularly flow-based intracellular cytokine staining and the Elispot assay, have allowed for more comprehensive and precise analysis of HIV-specific T-cell responses, which is vital to our understanding of the HIV-specific activity of CTLs. In a study published earlier this year in the *Journal of Virology*, Dr. Addo and her colleagues used an interferon- γ Elispot assay and 504 overlapping peptides spanning all HIV proteins, in order to

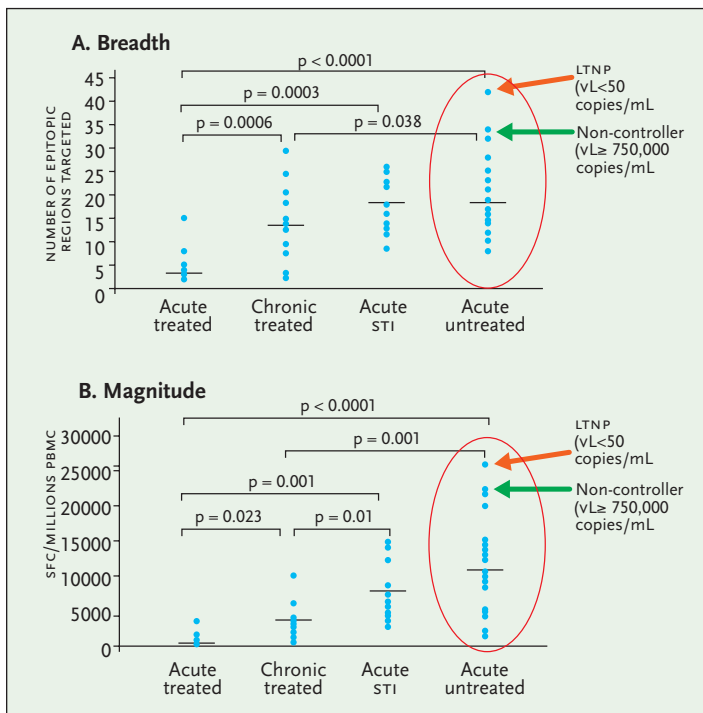


Figure 2. Breadth and Magnitude of HIV-Specific T-Cell Responses

Comparison of breadth and magnitude between the different study groups. Panel A summarizes the number of epitopic regions targeted per individual in the different study groups, while Panel B indicates the total magnitude of the CD8+ cell response to the entire expressed genome per individual (measured by Elispot and given in SFC/106 PBMC). No significant correlation to markers of disease progression—such as viral load and CD4+ cell counts—was observed. In the group of HIV-positive individuals with chronic, untreated infection, it was noted that the patient with the broadest and highest response was an LTNP with a viral load below 50 copies/mL. However, the patient with the second highest response had progressive HIV disease, with a viral load of more than 750,000 copies/mL.

Source: Marylyn Addo, MD, PhD. Adapted from: Addo MM, Yu XG, Rathod A, et al. **Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load** [Figure 4]. *J Virol* 77(3):2081-92, 2003.

evaluate the breadth and magnitude of HIV-specific T-cell responses in 57 HIV-positive individuals in various stages of infection (Addo, 2003). Of these 57 study subjects, 22 were diagnosed and treated during acute or early HIV infection—11 of whom received continuous HAART and 11 of whom had undergone one or two STIs—23 were chronically infected with HIV but were not undergoing antiretroviral therapy, and 12 were chronically infected with HIV but were undergoing therapy with HAART.

“We really had two hypotheses going into this study,” Dr. Addo said. “Our first hypothesis, if you are comparing people who control the virus and those who do very poorly and progress, we stipulated that HIV-specific cells responses in HIV controllers may target different regions of the virus than those in people who progress. Our second hypothesis was, well, maybe it’s a question of numbers. Maybe the immune response in controllers is much broader and much stronger than in HIV progressors.”

Dr. Addo’s team’s data demonstrated that all HIV proteins and protein subunits are targeted by HIV-specific CTLs, even in persons who failed to control HIV infection. “We also looked at the most frequently

targeted gene products in controllers and progressors,” Dr. Addo added. “Essentially, there was no difference between the two groups. In controllers, Nef, p24, and RT were the most frequently targeted proteins. And the same was true in the progressors.”

As for the breadth and magnitude of virus-specific T-cell responses, these varied significantly among individuals at different stages of infection, with the broadest and strongest responses detectable in individuals with untreated chronic infection and the more narrowly directed responses detected in treated acute and chronic HIV infection (see Figure 2). However, no significant correlation to markers of disease progression—such as viral load and CD4+ cell counts—was observed. “In looking at the group of HIV-positive individuals with chronic, untreated infection, we noticed that the patient with the broadest and highest response was an LTNP. This didn’t come as a surprise and we thought that this was exactly what we were looking for and that it fit our hypothesis nicely.” However, when Dr. Addo’s team looked at the patient with the second highest response, he turned out to be a patient with progressive HIV disease—he had a viral load of more than 750,000 copies/mL.

This lack of an inverse correlation between breadth and magnitude of HIV-specific T-cells—as measured by interferon-γ production—and viral load has been observed by several groups.

Assay Limitations

A POSSIBLE EXPLANATION FOR THE SOBERING RESULTS OF THIS STUDY CAN perhaps be tied to limitations of the assay used. As discussed by Dr. Addo, the sets of overlapping peptides used to assess virus-specific responses are based on sequences of primary HIV isolates or clade B consensus sequences. In turn, the use of such sequences is likely to favor detection of responses in well-conserved areas of the genome, since differences between the autologous virus sequence of an individual patient and the peptides used for the assays are smallest within these regions. For example, the proteins Vpr, Tat, and Rev are the most variable and were the least frequently targeted by CTLs. Conversely, the proteins that were targeted frequently—such as p24 and RT—were within more conserved regions of the virus.

To explore this observation further, Dr. Addo and her group conducted a study involving six individuals to systematically assess T-cell responses against p24, Tat, and Vpr peptides based on autologous sequences (Altfeld and Addo, 2003). The study demonstrated that 12/42 (29%) targeted peptides were only detected with peptides representing the autologous virus strain, compared to the HIV clade B consensus sequence. The use of autologous peptides also allowed for the detection of significantly stronger HIV-specific CTL responses in the more variable regulatory and accessory HIV proteins Tat and Vpr. Taken together, these data indicate that accurate assessment of T-cell responses directed against the more variable regulatory and accessory HIV proteins requires reagents based on autologous virus sequences. They also demonstrate that CTL responses to the variable HIV proteins are more common than previously reported.

“In this study, we only looked at three gene products,” Dr. Addo explained. “As the data suggest, the total magnitude and breadth was higher using the autologous sequences compared to the consensus sequences, particularly for Tat and Vpr. This difference may be even more pronounced if autologous peptides for the whole HIV genome are used. So we may need to know the sequence of the autologous virus to correctly assess breadth and magnitude of HIV-specific immune responses. However, this isn’t really feasible on a large scale, as it’s a very expensive and cumbersome process. But the point here is that this

may be a reason—potentially among others—why we and others may not have seen any correlations with HIV viral load in our earlier study.”

Looking for Correlates of Protection

DR. ADDO'S GROUP HAS CONDUCTED A NUMBER OF ADDITIONAL STUDIES to look for correlates of protection in HIV controllers. In one report presented at the 10th Conference on Retroviruses and Opportunistic Infections, Dr. Addo and her colleagues investigated a cohort of 50 HIV controllers—defined as HIV-RNA levels below 2,000 copies/mL in the absence of therapy—for a variety of possible correlates of protection (Addo, 2003a).

Given the fact that the magnitude and the breadth of the HIV-specific T-cell response were not different in HIV controllers compared to HIV progressors, Dr. Addo's team set out to perform preliminary studies on the quality of the immune response, specifically looking at the maturation status of HIV-specific T-cell responses. As explained by Dr. Addo: “Dr. Patrick Champagne [of the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland] proposed a lineage differentiation pattern of antigen-specific T-lymphocytes based on the expression of CD45RA and CCR7 antigens, suggesting a skewed maturation of memory HIV-specific CD8+ T cells with an accumulation of pre-terminally differentiated CD45RA-CCR7-cells in the peripheral blood during uncontrolled chronic infection.” Based on the hypothesis that viral controllers predominantly harbor HIV-specific responses of mature effector phenotype, Dr. Addo's group compared the effector phenotype of dominant HIV-specific T-cell responses in HIV. However, asserts Dr. Addo, these studies are preliminary and need to be confirmed on a larger number of study subjects.

Dr. Addo's group also looked at HLA alleles in these HIV controllers. It turned out that 60% of the HIV controllers had at least one allele associated with slower disease progression. Documented alleles included HLA-B57, HLA-B27, HLA-B14/Cw8, HLA-B51, and HLA-A25. In more recent work, Dr. Addo and her colleagues have evaluated individuals expressing the HLA-B57 allele with the hypothesis that they might have stronger CTL responses, lower and shorter peak viremia during acute infection, and would be less symptomatic during acute infection. “In two primary HIV infection cohorts, one in San Francisco and the other in Boston, we were able to find nine HIV-infected individuals expressing the HLA-B57 allele,” Dr. Addo explained. “Only three of the patients had symptomatic acute infection and all nine were maintaining viral loads below 5,000 without treatment, which is really quite striking.”


Turning their attention to the immune responses, Dr. Addo's group found that the responses in these nine patients were primarily being mediated by the HLA-B57 allele, while the other five HLA class I alleles expressed in each individual contributed less than 25% to the total HIV-specific T-cell response (a difference that did reach statistical significance). “We can conclude from this observation that these HLA-B57-positive individuals all had good control of primary HIV infection, were less frequently symptomatic during the acute phase of infection, and had strong and dominant HLA-B57-restricted immune responses during the acute HIV infection.” This indicates that long-term nonprogression may potentially “start” during the primary stages of HIV infection (Altfeld, 2003).

Another noteworthy finding in this cohort of 50 HIV controllers was that 50% of them tested positive for GB virus-C infection (GBV-C), a virus associated with decreased mortality and improved disease outcome in individuals infected with HIV (see our review of this particular subject in the September 2003 issue of *The PRN Notebook*). GBV-C infection was detected in approximately 20 (20%) of the HIV progressors. And while the difference GBV-C prevalence rates were not statistically significant—“this

analysis may not have had enough patients to yield statistically significant difference,” Dr. Addo said—the observed difference between the two groups warranted further exploration in a larger number of subjects.

HIV viral loads were significantly lower among GBV-C-positive individuals than among GBV-C-negative individuals. However, in the analysis of HIV-specific immune responses, the breadth and magnitude of HIV-specific CTL responses were not statistically different between GBV-C-positive and -negative study subjects. “There was a trend,” Dr. Addo said, “but nothing that really stuck out.”

Conclusion

WHAT REMAINS FOR DR. ADDO AND HER GROUP AT PARTNERS AIDS Research Center are a lot of questions. “We know that broad and strong HIV-specific CD8+ cell responses are detectable in both controllers and progressors,” she said in her concluding remarks. “But as we've shown recently, the differences in the immune response may not simply boil down to quantitative issues—not just a numbers game—but rather issues of function and quality. It's possible that HIV-specific T-cell function in HIV controllers is better than the one in HIV progressors, in ways that we have not yet fully determined. There are many genetic, immunological, and virological factors that may lead to improved disease outcome in HIV controllers. Our data suggest that HIV controllers are a very heterogeneous group in terms of the individual factors contributing to viral control. We need to continue exploring these factors and the relative impact they may have.” 

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