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Elite HIV Controllers: Myth or Reality?

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Abstract

Despite the varying disease progression rates, the majority of HIV-infected individuals eventually progress to AIDS. There is a subset of HIV-positive individuals, who maintain high CD4+ and CD8+ T-cell counts, remain therapy naive and persistently infected with HIV-1 for more than 15 to 20 years. In light of current observations, this subset can be divided into two groups. One shows low detectable plasma viremia (< 5000 HIV-RNA copies/ml), termed long-term nonprogressors. A second group shows plasma HIV-RNA values persistently below 50 copies/ml throughout the course of infection, and termed "elite" or "natural controllers". The features common between both groups are the presence of high CD4+ and CD8+ T-cell counts, strong immune responses, and low but variable cellular proviral DNA load. The group of HIV-positive long-term nonprogressor individuals comprises about 1% of the total HIV population in the world, whereas the "elite" controllers may be much less. Why do some people deteriorate faster, while others remain normal both symptomatically and immunologically for decades? There is a renewed interest in HIV-positive individuals who have survived since the period close to the earlier part of the HIV pandemic in the 1980s and have remained drug-naive. As very little is known about "elite" controllers, the findings discussed here are largely based on previously known and newly emerging aspects of HIV pathogenesis in the context of the long-term nonprogressor group. It is believed that data emerging on long-term nonprogressors will allow us to make scientific inferences to further our research on "elite" controllers. Aspects dealing with cellular, humoral, innate, and adaptive immunity, which are relevant to nonprogressive HIV disease, are beyond the scope of this review. **(AIDS Rev. 2007;9:195-207)**

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Key words

HIV. Long-term nonprogressors. Elite HIV controllers.

Introduction

"It is too early for optimism, but the research on HIV elite controllers could be promising. I believe we should get out and study elite controllers, but we should be conservative in what we expect from this research", said recently Anthony Fauci (San Francisco Chronicle, 8/17/2007).

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HIV/AIDS continues to be one of the most significant infectious diseases globally. According to new UNAIDS data the global HIV infections have been estimated at 33.2 million people living with HIV as of 2007, 2.5 million new infections occurred in 2007 and 2.1 million people died of AIDS in 2007 alone (2007 AIDS Epidemic Update/ Global Summary, UNAIDS).

Both viral and host factors are known to influence the clinical course of HIV disease. This clinical course is variable between different human hosts and is characterized by a gradual loss of $CD4^+$ T-cells and cellular immunity concomitant with increases in plasma viremia, which eventually results in the development of AIDS. Usually in HIV patients, AIDS develops typically within 8-10 years in those who do not receive therapy, but with the advent of highly active antiretroviral therapy (HAART) morbidity

and mortality rates have dramatically fallen and HIV-positive patients have a better quality of life. Variable disease rates are seen among HIV-infected individuals, with rapid and slow disease progression in some individuals, whereas completely asymptomatic phases of more than 15 years may also occur rarely in a subset of individuals who otherwise maintain a healthy immune system, CD4/CD8$^+$ T-cell counts, undetectable viremia, and remain therapy naive throughout the course of HIV infection.

Despite the multitude of both viral and host factors known in the context of HIV disease progression, very little is known about natural clearance of HIV in adults and complete natural control of HIV-1 in human hosts. The natural or elite controllers (a small subset of previously diagnosed long-term nonprogressors, LTNP) harbor below-detectable plasma viremia (< 50 copies/ml) as opposed to the LTNP who show variable but low plasma viremia. Goudsmit, et al. in 2002[1] have shown in the Amsterdam cohort that 95% of HIV-positive individuals, who were consistent with the definition of LTNP, have either progressed to HIV disease or have shown signs of progression, which is consistent with studies on members of the Sydney Blood Bank cohort who now are categorized as long-term survivors, despite harboring *nef* and *rev* attenuated viral quasispecies[2-5].

It is now beginning to emerge that approximately 1% of all LTNP show persistent HIV infection, which they are able to control naturally with the help of unknown immune factors in the absence of therapy. These are "elite or natural controllers" who are therapy naive HIV-positive individuals and who remain infected with HIV and whose bodies have kept the HIV at below detectable levels in their blood for 15-20 years or more. Overall, these individuals have been largely invisible to the scientific community, partly due to their healthy HIV disease status, and have not required any drug treatment, and hence had minimal contact with the health system. Therefore, these individuals have never qualified for further clinical investigations and have remained obscure. Twenty-four years have elapsed since the discovery of HIV and several such HIV-positive individuals continue to survive since the early part of HIV epidemic. Simply because of their drug-free survival with HIV over two decades, this has generated a renewed interest in this group of individuals. These individuals can provide clues to natural therapeutics and possible clues to therapeutic vaccines for HIV. This review embarks on discussing various aspects of HIV pathogenesis in the context of previously known and currently emerging host/viral aspects of HIV pathogenesis in this unique group of HIV-positive individuals. The aim of this review is to discuss aspects in the context of natural control of HIV disease and provide stimulating thoughts for future work on HIV/AIDS in this very important area of biomedical research.

Unifying features of nonprogressive HIV disease

So far, most studies done on nonprogressors to investigate natural control of HIV infection have been done on small subsets of patients. Through observations it is beginning to emerge that nonprogressive HIV disease should now be classed into two therapy naive subsets, based on persistent low-level detection of plasma viremia in LTNP as opposed to sustained, below-detectable levels of plasma viremia in natural or elite controllers for 15-20 years or more. For now, this is probably the only difference that segregates LTNP from elite controllers in want of other unknown host and viral factors. It is unclear whether the resistance to immunologic damage by HIV in this group of nonprogressive individuals will last indefinitely, or whether it merely represents the extreme end of Gaussian distribution and eventually these individuals will progress to AIDS[6]. Nonetheless, the research efforts spent on this group will definitely unveil the unifying host-genetic and viral traits that underlie nonprogressive HIV disease.

To date, most studies have confirmed a strong relationship between plasma viral load, stable CD4$^+$ T-cell counts, and long-term nonprogressive HIV infection[6-9]. Thus, low plasma viral load and possibly low cellular proviral load along with stable CD4$^+$ T-cell counts may be one of the most important unifying features segregating LTNP and elite natural controllers. Kloosterboer, et al.[10] identified and studied four unique therapy naive individuals seropositive for HIV-1 antibodies, but who showed extremely low levels of proviral DNA and undetectable levels of plasma viremia. Three of these four individuals harbored HIV antibodies for more than 13-22 years. The HIV could not be isolated, despite using large quantities of patient peripheral blood mononuclear cells (PBMC), which appears to be consistent with several other previous studies[8,11]. Several attempts were made to isolate replication-competent virus from these subjects, but the replication-competent virus could neither be isolated using higher concentration of T-cells nor from the purified CD4$^+$ T-cells, which agrees with previous studies[12]. Or, if successful isolation of virus was achieved, the viruses from such individuals systematically displayed slower replication kinetics *in vitro*[13]. All individuals expressed protective human leukocyte antigen HLA-B*58s alleles and showed evidence of HIV-specific cellular immunity either by staining with HLA-B*57 tetramers folded with an HIV reverse transcriptase (RT) or gag

peptide, or after stimulation with HIV-1 p24 gag, RT, or nef peptides in ELISPOT analysis. The HIV-specific $CD4^+$ T helper cells (Th) were demonstrated by proliferation of $CD4^+$ T-cells and intracellular staining for interleukin-2 (IL-2) and interferon gamma (IFNγ) after stimulation with an HIV gag peptide pool, which is consistent with a previous study by Zaunders, et al.[14]. Sera of all individuals showed antibody mediated neutralization of both R5 and X4 HIV-1 variants.

Recently, Lambotte, et al.[9] have analyzed data of 15 patients from a cohort of 1300 patients from France, which they referred to as "HIV controllers". They also found very low and very stable HIV proviral DNA loads in PBMC in all 15 patients. These low HIV DNA loads contrast with the amounts observed in patients receiving prolonged and efficient HAART[15]. Their findings help explain the lack of disease progression despite prolonged infection because the HIV DNA load has a significant and independent influence on the rate of disease progression[16]. In addition, as observed previously[8,10,14], patients in Lambotte's study[9] were also characterized by strong HIV-specific immune responses. Together, these data implicate that very low-level antigen exposure is sufficient for sustained HIV-specific immunity, and suggest the possibility of a multi-factorial control of HIV infection, fully consistent with other studies[8,14]. In all these studies, it is notable that the natural controllers could be distinguished from LTNP by extremely low levels of proviral DNA, below-detectable levels of plasma viremia, and poor viral replication *in vivo*, a phenomenon consistent in this group of HIV patients and with earlier studies[17,18].

Furthermore, viral phenotype is also critical in progressive and nonprogressive HIV disease. At the level of viral tropism we already know that the dual tropic (which can use both CCR5 and CXCR4) and the CXCR4-using HIV strains appear to be more prevalent during rapid progression of HIV disease[7,19-21], whereas there is a preponderance of less cytopathic and CCR5-using HIV-1 strains during nonprogressive HIV disease, a phenomenon analogous to the selection/emergence of CCR5-using strains during aviremic states on HAART therapy[22,23].

Viral attributes of HIV disease

HIV replication, plasma viral load, viral evolution and nonprogressive HIV disease

Previous work has shown that viral factors such as plasma viral load[24], enhancement of viral transcription/replication[25-27], prevalence of cytopathic HIV strains[28], and infection with attenuated HIV-1 strains[29-31] have been shown to play a pivotal role at different stages of HIV disease.

It has been shown that the host-induced control of HIV-1 replication in LTNP could be attributed to plasma viral loads and proviral DNA load in peripheral blood cells, which are several orders of magnitude lower than what is seen in progressive disease[32]. Because of such low abundance of viral copies in LTNP, often the isolation of HIV strains from these individuals has proven difficult *in vitro*. This also correlates with low copies of HIV provirus in cells of aviremic nonprogressors, along with high T-cell counts[33]. This obvious control over HIV has been hypothesized to be due to variable degrees of virus trapping in the follicular dendritic cell network in the lymph nodes, especially in HIV-positive individuals with nonprogressive HIV disease, which paralleled the extent of lymph node germinal center formation. Further, it has also been hypothesized that lower quantity of circulating virus may be reflected in the lower degree of virus trapping in association with the decreased rate of tissue activation as observed in LTNP with low viral loads[34].

Lower multiply spliced RNA15 has been also reported in LTNP, which is consistent with those of a large number of published reports, suggesting the rate of disease progression is driven by an increasing viral burden[32-34]. Through the multicenter hemophilia cohort study it has also been revealed that HIV-1 RNA levels during early chronic HIV-1 infection is a strong age-independent predictor of clinical outcome, and low levels of plasma virus define persons with a high probability of long-term AIDS-free survival[8-10,18,35.] Recently, Bello, et al.[36] determined if natural suppression of plasma viremia below the detection limit of commercial assays (HIV-1 RNA 50-80 copies/ml) can contain the HIV-1 evolution. They assessed HIV-1 *env* gene quasispecies complexity in PBMC DNA at two time points in 14 LTNP. Sequence changes were consistent with viral evolution in all patients with a median plasma RNA viral load > 100 copies/ml. There was evidence of low-level viral evolution in two of four patients with intermittent viremia and a median plasma HIV-1 RNA load of > 80 copies/ml. No significant evolution was observed in the three LTNP with persistent viral suppression below the detection limit, a phenomenon consistent with observations of Wang, et al.[8]. Under these conditions, it is apparent that very low-level HIV antigen probably is sufficient for sustained immunity**.** Overall, a significant positive correlation ($p < 0.001$) was observed between viral evolution and plasma RNA viral load in the LTNP analyzed. While measurement of plasma viremia offers important prognostic information, the underlying viral factors responsible for prolonged control of viral replication *in vivo* in LTNP remains as yet unclear.

Viral variability, diversity and HIV disease rates

There is evidence to suggest that the HIV disease progression is a consequence of the attainment of a threshold level of viral antigenic diversity above which the host immune system is nonresponsive[37]. Loss of immune containment and triggering of disease progression have also been attributed to clonal dominance or deceptive imprinting of the immune response towards the virus established in infection. This is partially aided by possible saturating levels of circulating viral antigen[38]. Viral genetic diversity has been shown to have a profound influence on HIV disease development and on rates of disease progression[7,39,40]. The overall accumulation of viral genetic diversity is slower in individuals who progress rapidly to AIDS. In other words, the homogeneity in viral quasispecies or clonal dominance determines the progressive HIV disease[7,40,41]. In contrast, higher accumulation of genetic diversity and increases in both synonymous and non-synonymous site substitutions is seen over time in infected individuals who either progress slowly or do not progress at all (nonprogressors)[7,39,40]. Although both non-synonymous and synonymous site substitutions increase over time in slowly or nonprogressive patients, critical differences have been observed between patients with diverse rates of disease progression[7,39]. Thus, the HIV-infected nonprogressor and slowly progressing individuals are characterized by a higher accumulation of non-synonymous base substitutions (that typify positive selection), suggesting that higher genetic diversity is the determinant of slow progression and/or nonprogression in HIV disease. A recent study by Ross and Rodrigo[42] has further shown that the broad genetic diversity of HIV-1 in an infected individual is a result of site-specific positive selection for diversity, a likely consequence of immune recognition. As suggested by this study, the positive selection appears to be a good indicator and predictor of duration of HIV disease. This positive selection in long-term progressors persists over time and appears to be associated with helper T-cell epitopes. In contrast, sites under positive selection shift from one time point to another in normal progressors. Thus, a broad and persistent immunologic response is associated with a slower rate of disease progression or nonprogressive HIV disease[11,42]. In contrast, individuals who mount a limited and shifting immunologic response to HIV have fewer and less persistent positively selected sites, and progress more rapidly to AIDS. Based on the *vpr* genes derived from an epidemiologically linked cohort of three individuals (a nonprogressor, with 20 years of HIV infection and therapy naive transmitter, and two recipients who progressed to AIDS upon transmission of HIV from the nonprogressor), Cali, et al.[45] have shown that Vpr quasispecies is positively selected in two progressing recipients and not in the nonprogressor patient. Two studies by Mikhail, et al.[45,52,] independently confirm that the viral evolutionary rate and the positive selection of viral quasispecies at the level of full-length genome and individual genes (such as *vpr* and *tat*)[53] may be the actual determinants of HIV disease rather than single amino acid substitutions.

Although not a unifying feature of all nonprogressor HIV-positive patients, some LTNP appear to be infected with attenuated HIV strains and this attribute only partially controls HIV replication via possible impaired replication kinetics, resulting in low plasma viremia and hence delay in disease progression[21,30,43-50]. These attenuations were seen in both structural and regulatory genes of HIV. Most of the aforementioned studies are based on analysis of Nef, Tat, Vpr and Rev gene mutations and none of the studies have provided a single mutation in the regulatory/accessory genes to be commonly relevant to progression or nonprogression of HIV disease.

The highly variable envelope gene is one of the best to interpret viral changes in relation to host-selection pressures *in vivo*. Previously higher env peptide variability (3-21%) has been seen in HIV strains derived from LTNP and slow progressors, as opposed to normal progressors[7]. Wang, et al. reported a unique V2 region extension that was identified only in slow and nonprogressors[7]. The functional role of this V2 region extension in the same set of patients was recently confirmed to be in the maintenance of CCR5 usage over time in both slow and long-term nonprogressing HIV disease[21]. These data imply that certain gene changes, similar to that seen in the *env* V2 region, may play a pivotal role in favoring the selection of less pathogenic viral variants leading to slow and/or nonprogressive HIV disease. Roman, et al. identified uncommon amino acid substitutions in the V3 loop regions of HIV-1 strains obtained from infected patients from Rwanda. The frequency of these variations was greater in LTNP compared with late-stage patients ($p = 0.006$), particularly in a sequence region that has crucial interactions with the cell surface, and is highly relevant for the host's immune response[51]. These variants might reflect a viral response to a strong immune pressure, or represent attenuated HIV-1 strains infecting LTNP[51]. Supporting this, another recent study by Wang, et al.[8] provides a compelling documentation of the presence of several stop codons appearing in the p17 and p24 region of the *gag* gene, possibly contributing to the complete lack of viral evolution in one unique "true nonprogressor"[8]. This is further discussed in the light of hypermutation in the next section.

Despite the overwhelming literature available on all the diverse occurrences of HIV-1 mutations at different stages of HIV disease[8,45,48,52-54], all the studies have failed to provide correlating markers of disease progression or nonprogression at the level of single substitution or motif. This could be attributed to strain differences in HIV-1 strains and their evolution *in vivo* in conjunction with the host. Thus, as a consequence of differential host selection pressures imposed on viral quasispecies *in vivo*, the single amino acid variations are different and will differ between patients. In summary, the host has a strong contribution in guiding positive selection of HIV *in vivo*[45]. It should be emphasized that these single amino acid changes are valid for interpreting disease in a given patient, but they cannot provide global interpretation of such changes.

G→A hypermutation and resistance to HIV disease

The previous section has discussed viral variability and mutations in the context of HIV disease, but there is a specialized set of mutations, such as hypermutation, which needs special discussion in relation to HIV disease. Hypermutation, which involves excessive G→A substitutions in the dinucleotide context GA or GG, has been sporadically observed in HIV-1. A retroviral provirus is dubbed a "hypermutant" if it undergoes an inordinate number of identical transitions (usually guanine→adenine). Hypermutation usually results in the production of replication-incompetent virus due to the introduction of new stop codons. Hypermutation was first found during propagation of HIV-1 *in vitro*. However, since then several groups have reported the isolation of hypermutated HIV-1 sequences from clinical samples[55-60]. Most of the studies of hypermutation caused by APOBEC3 have been done in viral cultures. The APOBEC3G, is a host cell cytidine deaminase that in the absence of HIV-1 Vif is capable of inducing extensive gene hypermutation through which viral replication is considerably attenuated.

Janinini, et al.[57] sequenced protease genes from the PBMC of 53 HIV-1-positive patients infected with subtypes A through D at different clinical stages. These researchers found that 43% of patients harbored hypermutated, along with normal, protease genes. Another group[58] also studied the presence of hypermutation on the reverse transcriptase and protease genes in proviral DNA from resting CD4 T-cells from patients who had prolonged suppression of viremia on HAART. They found that hypermutated sequences constituted > 9% of the viral genomes in this compartment and were found in every patient. The sequence context of the G→A changes were consistent with the known site preferences of APOBEC3G and APOBEC3F, suggesting that these enzymes can be responsible for G→A changes *in vivo*. It is logical to hypothesize that low levels of APOBEC3G may lead to G→A sustaining evolution and viral escape mutations, but excessive actions of APOBEC3G may render HIV genomes non-replicative. To support this, a case study[61] showed a subject confirmed HIV-1 positive in June 1995. The patient did not receive any antiretroviral therapy, had no AIDS-defining illness at the time of his plasma collection, and had a plasma viral load of 49,000 copies/ml, and $CD4^+$ and $CD8^+$ cell counts of 456 and 538 cells/l blood, respectively. Three HIV full-length genomes from this individual's genomic DNA showed that the viruses had intact open reading frames and belonged to subtype B. Hypermutation analysis illustrated the diverse G→A hypermutation rates in different open reading frames when HXB2 was used as a reference strain. A high G→A hypermutation rate was found in the HIV-1 *pol* and *vif* regions, and revealed that the G→A hypermutation induced premature stop codons in the genome: eight in the *pol* gene and two in *vif*. Interestingly, 10 stop codons were uniformly caused by the G→A transition, converting the amino acid tryptophan to the terminal signal (amino acid position: protease 42, RT51 88, RT51 212, RT51 398, RT51 410, RT51 414, RNaseH 95, integrase 132, Vif 70, Vif 89), as is characteristic of APOBEC3G enzyme[62,63]. Wei, et al.[61] found that the biased G→A hypermutation, concentrated in *pol* and *vif*, was not caused by the imbalanced distribution of G, GA and GG nucleotides, but may have been caused by endogenous APOBEC3G activity.

In 2003, Wang, et al.[8] also demonstrated a case of a 55-year-old, male LTNP from Sydney, Australia. Over time analysis of full HIV genomes showed the first evidence for non-evolving HIV genomes. They were highly intact and displaying extensive hypermutation in the *gag-pol* region only (and not in Vif), with the *gag-pol* gene interrupted by five major stop codons in the RT, protease and p24 regions. This phenomenon has never been observed in progressing patients. Measurement of APOBEC3G levels in peripheral blood cells from this LTNP and control progressor failed to reveal significant difference in levels of APOBEC3 protein *in vivo* and a direct relationship with hypermutation (unpublished data). In light of our data and that of Wei, et al.[61] it is believed that hypermutation tends to occur in sequences from LTNP inducing pre-mature stop codons, but it can also be found occasionally in sequences from progressors. It remains unclear how APOBEC3G functions in relation to these observations. Nevertheless, these findings do support that the hypermutated HIV genomes evolve slowly and, as a result of

G→A hypermutation-induced damage, HIV genomes are rendered incapable of replication[8]. The latter occurs only in a fraction of individuals, but may have therapeutic implications.

Together, these data raise an interesting possibility that polymorphisms of APOBEC3G may also be associated with HIV progression. An, et al.[64] studied APOBEC3G genetic variants and their influence on the progression to AIDS. They genotyped APOBEC3G from 3073 HIV-positive subjects and investigated the correlation between APOBEC3G genotypes and the progression to AIDS. Their findings show that the APOBEC3G haplotypes were distributed differently among individuals of Asian, European, and African ancestry. They found that the presence of 186R/R genotype was significantly associated with rapid progression to AIDS and death in African Americans. However, the antiviral activity of the 186R enzyme was tested *in vitro*, and failed to show any difference compared to the common H186 variant. It would be interesting to determine if the 186R genotype is also associated with increases in G→A mutations in the HIV-1 genome and suggests that the mechanistic pathway *in vivo* may differ from the ones known from several *in vitro* analyses. Although a number of studies have provided evidence for correlation between HIV infection and hypermutation, the actual clinical relevance of hypermutation and its contribution to HIV disease remains unclear.

Viral levels and APOBEC3

A variety of mechanisms of innate immunity that protect organisms from retroviral infections, including HIV, are known. Lentiviruses express viral infectivity factor (Vif) protein that has the ability to counter antiviral activity exhibited by the recently discovered host cytidine deaminases APOBEC3G and 3F. Although these host factors are present in diverse mammalian species and have been shown to act against various organisms, their importance in HIV infection has been highlighted because of their suggested activities against HIV *in vivo* and the strong conservation of the HIV *vif* gene encoding the Vif protein capable of countering this innate activity.

Jin, et al.[65] have recently published the first study correlating the level of APOBEC3G and two parameters of HIV progression. They measured HIV viremia, CD4 count, and the level of APOBEC3G mRNA in PBMC from six HIV-uninfected and 25 HIV-infected subjects (8 LTNP and 17 progressors). Although none of the *in vitro* studies have demonstrated the role of HIV in directly regulating APOBEC3G mRNA levels, Jin, et al.[66] found an inverse correlation between APOBEC3G mRNA levels and HIV viral load, and a highly significant positive correlation between APOBEC3G mRNA levels and CD4 cell counts in those patients. Furthermore, they found that the level of APOBEC3G mRNA is different in the three groups: LTNP > HIV-uninfected > progressors. These results are very important as they show a direct relationship of APOBEC3G levels and two predictors of HIV disease progression in patients naive of treatment. The higher level of APOBEC3G in LTNP may indicate a role of this protein in the nonprogression of HIV disease. It would have been very interesting to also have the sequence analysis of the HIV genes in these patients to correlate the presence of hypermutation with the level of APOBEC3G and HIV viremia. In contrast, a recent publication[66] failed to detect an association between these parameters and no relationship between APOBEC3G/3F mRNA levels, plasma viremia and CD4 counts. Thus, these studies are preliminary and need an in-depth confirmation of the role of APOBEC3G/3F in nonprogressive HIV disease.

Influence of other viral agents on HIV infection and disease progression

Persistent viral infections other than HIV-1 in the HIV-positive individuals can act as important cofactors affecting the course of HIV disease. Some viruses have been shown to have a deleterious effect on HIV-1 progression by increasing its replication. The mechanisms implicated include an increase in counts of activated $CD4^+$ T-cells, an increase in proinflammatory cytokine levels, and an elevation in immune marker levels. Recent studies, however, suggest that certain pathogens may have the ability to attenuate HIV-1 infection and reduce disease progression. These include GB-virus C (GBV-C) and human T-lymphotropic virus type 2 (HTLV-2). In a different manner, HIV-2 infection has shown delayed disease progression in most people, although the possible protective effect of HIV-2 over HIV-1 infection is controversial.

The GBV-C is a single-stranded RNA virus, member of the *Flaviviridae* family and related to hepatitis C virus (HCV). However, unlike HCV it is not hepatotropic and has no clear association with a disease state. Infection with GBV-C is relatively common in HIV patients, with a prevalence of over 30-35%[67]. It replicates in lymphocytes, and coinfection with HIV-1 seems to improve morbidity and mortality for the HIV-infected individual. This beneficial effect has been described in several cohort studies[68-70]. *In vitro* studies have also shown the inhibition of HIV replication by GBV-C[71,72]. The mechanisms by which GBV-C benefits HIV infection remain obscure, although it seems that GBV-C infection leads to stable serum levels of T-helper 1

(Th1) cytokine along with increased IL-2, IL-12, and INFγ levels. In addition, it causes a downregulation of Th2, IL-4, and IL-10 cytokines. This specific cytokine profile seems to improve the HIV outcome[73]. Furthermore, GBV-C decreases expression of CCR5 and CXCR4 HIV coreceptors by inducing specific chemokines[74] and it is also thought to decrease the transcription of HIV from integrated provirus[75]. However, there is still some controversy regarding this interaction, and other reports are more cautious regarding the potential benefit on HIV infection[76,77]. On the other hand, GBV-C does not seem to play any role in HCV infection, and although HCV and hepatitis B virus (HBV) seem not to affect the course of HIV disease in coinfected patients, there is little doubt that they can account for an increased risk of liver disease and hepatotoxicity related morbidity and mortality in these patients[78].

The HTLV-2 is another virus that appears to attenuate HIV disease progression. Some reports showed evidence that coinfected patients had higher $CD4^+$ T-cell counts, with slower decline rates and lower HIV RNA levels improving survival and delaying progression[79,80]. This protective effect may be the result of lowering HIV replication and modulation of immune activation. The HTLV-2 Tax protein might have an immunomodulatory effect, increasing the production of IFNγ[81]. It has been suggested that HTLV-2 infection induces production of CCL3, CCL4, and CCL5 chemokines, which are natural ligands of CCR5, resulting in the inhibition of HIV replication. Recently, it was further demonstrated that the upregulation of CCL3L1 observed in HTLV-2/HIV-1-coinfected persons was a potent inhibitor of R5 HIV-1 strains[82]; in addition other chemokines and cytokines induced by HTLV-2 seem to favor the Th1 response instead of the Th2 response involved in disease progression. Other studies have shown that HIV Gag-specific cytotoxic T-lymphocyte (CTL) responses were dominated by single CCL4-producing $CD8^+$ T-cells in HTLV-2/HIV-coinfected persons, supporting the hypothesis of a protective effect[83]. Whether HTLV-1 infection exerts a similar benefit is unclear and at present it appears to be exclusive of HTLV-2.

It has also been suggested a protective effect of HIV-2 infection over HIV-1. However, this hypothesis has remained controversial and the most recent epidemiologic data do not support it[84,85]. Moreover, the degree of disease progression in HIV-2/HIV-1-coinfected patients is largely undefined, although it might be similar to HIV-1-monoinfected patients[6]. The HIV-2 infection by itself is considered a natural model for attenuated disease; most HIV-2-infected people fit in the characteristics of LTNP and live a normal lifespan. This difference in disease outcome with HIV-1 might be explained by lower plasma HIV-2 RNA levels. This suppression of viral replication may be due either to viral factors, host restriction mechanisms, or immune control; the literature however, is scarce on these issues. Recent evidence suggests that many HIV-2 isolates have a lower replicative capacity than HIV-1 group M isolates[86], which could explain the low viremia. An alternative explanation for this control of viremia is the stronger and broader immune responses observed in HIV-2 patients. Specific CTL responses appear to play an important role, the quantity and quality of which seems more robust in HIV-2-infected patients with higher Gag-specific T-cell responses[87]. But, most important seems the $CD4^+$ T-cell response, superior both in magnitude and function, supporting a strong specific Th response[88]. However, it is still not clear whether this strong immune response is primarily responsible for the control of viremia, or a consequence of this reduced viremia, which preserves the immune system. A recent report proposes the HIV-2 Env as a key factor that might modify immune responses. The study suggested an immunosuppressive effect of HIV-2 envelope limiting bursts of T-cell activation by acting on monocytes to hamper T-cell stimulation, resulting in a reduction of viremia and thus contributing to slow disease progression[89]. Despite these many unsolved questions, HIV-2 infection provides unparallel opportunities to understand HIV disease pathogenesis.

Rare events defining nonprogressive HIV infection

Nonprogressive HIV disease itself is a rare event and, therefore, sometimes virologic/biological information collected from individual cases of HIV-infected individuals can be very informative in understanding resistance to AIDS[8,14,30]. One such example is of a recent case from Australia of an HIV-positive LTNP, whose infecting strain has never evolved and the underlying reasons have been discussed in other sections above[8]. Currently available data agrees with this, and the LTNP has been shown to harbor slowly evolving genome characterized by very little evolution of HIV genomes. Further, the $CD4^+$ T-cells from this individual are resistant to the cytopathic effect incurred by HIV on T-cells, as shown by infection of $CD4^+$ T-cells by monotropic and dual tropic HIV-1 strains[11].

Another interesting example is of an LTNP 135 from Australia, who has been infected for the last 25 years. He is a part of *nef*-deleted Sydney Blood Bank Cohort (SBBC) described in 1994[3]. Recently, Zaunders, et al. have shown[90] that this individual has cleared the integrated proviral DNA for which he tested positive in 1994. In addition, this individual has maintained below-detectable

levels of plasma viremia since 1994 and no culturable virus has been isolated despite several attempts. In literature, this is the first report of an HIV-positive adult who appears to have cleared the integrated HIV proviral DNA as shown by ultrasensitive qualitative and quantitative PCR assays. The individual is CCR5 heterozygous and has HLA-B57 genotype, consistent with some protection against HIV disease. He has tested consistently weak for anti-p17, anti-p24 and anti-Gp120 antibodies by western blot, unlike other HIV-positive LTNP who had complete responses. In contrast to the humoral response, patient 135 had vigorous CD4+ and CD8+ T lymphocyte proliferative responses to p24 and gag peptides *in vitro*. Thus, this individual may be one of the rare natural controllers belonging to extreme end HIV patients. Nonetheless, such patients provide clues that some hosts may have mechanisms which can clear the virus, and the *nef* deletions in HIV may have a lesser role in the SBBC cohort 1, as in most SBBC members the *nef* deletions increased in size and some members progressed to HIV disease[5].

Newly emerging concepts relevant to nonprogressive HIV disease

New surface markers and other biomarkers in nonprogressive HIV disease or resistance to AIDS

HIV is known to infect practically every type of blood leukocytes and each of these leukocytes has a different immunologic role in combating viral infection. The HIV infection leads to characteristic alterations in the subset composition of circulating CD4+ and CD8+ T lymphocytes and several other blood leukocytes. The activation marker CD38, in particular, and its level of expression on CD8+ T-cells, is a marker that is strongly associated with immune activation, particularly during primary HIV-1 infection and progression to AIDS, respectively[91-93]. Furthermore, decreased expression of CD38 on CD8+ T-cells is highly correlated with the effectiveness of antiretroviral therapy[93-97], and lack of activation and expression of CD38 and HLA-DR on CD4+ T-cells correlates with long-term nonprogression[93]. The enumeration of CD4+ T lymphocytes by flow cytometry is used routinely in the clinical management of HIV-infected individuals to monitor the severity of immunodeficiency caused by HIV, and this acts as a basis for commencing HAART and prophylaxis for *Pneumocystis carinii* pneumonia[98]. As both CD4+ and CD8+ T-cells play an important role during HIV pathogenesis, it is believed that more information on the progression to immunodeficiency may be found in the detailed subset composition of CD4+ and CD8+ T-cells, but this is currently restricted to research studies.

To date, the immunophenotyping of CD antigens relies on flow cytometry. Although very reliable, the flow cytometry only allows estimation of 3-6 markers in a given assay. The recently developed antibody microarray DotScan™ technology enables the simultaneous analysis of a large number of cell surface antigens on a single chip. This new technology may permit the identification of novel differential markers expressed or co-expressed on CD4+ and CD8+ T-cells, which could aid in defining the stage of evolution of HIV infection and the immune status of the patient[99,100]. The assessment of CD markers in relation to HIV disease state is not new, but their assessment in the context of different blood leukocytes in predicting HIV disease stages is novel. Wu, et al.[100] have recently shown through analysis of 135 surface antigens on CD4+ and CD8+ T-cells using antibody microarray that based on CD markers on different cell types, HIV disease stages can be predicted. At the level of CD4+ T-cells, they showed that the LTNP-CD4 differed from BDL and/or VIR groups in that CD71, HLA-DR, CD38, CD3ε and CD183 were significantly upregulated. With regard to cell signaling, CD3ε expression on CD4+ T-cells was found to be significantly lower in the VIR group than in LTNP, which indicates that the expression level of CD3ε can be used to differentiate VIR and LTNP groups. This is the first time that the CD3ε has been found to have a relationship with HIV disease status and the biological significance of CD3ε expression in HIV disease still requires further elucidation.

In contrast, at the level of CD8+ T-cells, the LTNP differed from those therapy experienced with below-detection plasma viremia and the viremic groups in that CD8+ cells showed higher expression of CD11c, CD16, and CD56, all differences being statistically significant except CD56 in the LTNP-BDL comparison ($p = 0.0871$). Interestingly, the expression levels of two natural killer-associated receptors on CD8+ T-cells, CD16 and CD56 were significantly higher for LTNP than for BDL and VIR groups, though not all detected differences reached statistical significance ($p = 0.0093$-0.0871). Previous study has shown that CD56 is expressed on a subset of CD8+ T-cells (mature cytolytic effector cells), and the defective expression of CD56 on these cells in HIV-infected individuals could contribute to the decreased peripheral blood T-cell cytotoxicity found in HIV infection[101]. These findings, together with ours, support that the CD8+ T-cells from LTNP may have stronger cytotoxic activity than those from other HIV-positive individuals.

This is the first study to show clear differences on the cell surface of two distinct T-cell subsets at various stages

of HIV disease, and significant association of the expression of distinct cell surface antigens uniquely in nonprogressors. Further biological analysis is warranted on a larger patient subset to define their relevance and their actual role in nonprogressive HIV disease.

Human gene expression studies on distinct cell subsets and HIV disease

The HIV-1 alters gene expression in infected cells, leading to cellular dysfunction. Sheeter, et al.[102] and Corbeil, et al.[103] uncovered a number of host cell genes that are modulated in both $CD4^+$ T-cell lines and primary $CD4^+$ T lymphocytes infected with HIV-1, using high-density oligonucleotide probe microarray technology. Although changes in T-cell function and alterations, over time, is a characteristic feature of HIV infection, the underlying pathogenic mechanisms and the expression of gene machinery in distinct cell subsets during HIV infection remains unknown. These mechanisms can provide leads to the host genetic attributes of HIV disease and the reasons for progressive and nonprogressive HIV disease. Recently, Hyrcza, et al.[104] have examined the gene expression profiles in *ex vivo* human $CD4^+$ and $CD8^+$ T-cells from untreated HIV-1-infected individuals at different clinical stages and rates of disease progression, and show that HIV-1 infection induces characteristic patterns of gene expression in $CD4^+$ and $CD8^+$ T-cells and that these patterns can distinguish progression from nonprogression. Profiles of pure $CD4^+$ and $CD8^+$ T-cell subsets from HIV-1-infected nonprogressors with controlled viremia were indistinguishable from those of individuals not infected with HIV-1. Similarly, no gene clusters could distinguish T-cells from individuals with early infection from those seen in chronic progressive HIV-1 infection, whereas differences were observed between uninfected individuals or nonprogressors versus early or chronic progressors.

In early and chronic HIV-1 infection, three characteristic gene expression signatures were observed: (i) $CD4^+$ and $CD8^+$ T-cells showed increased expression of IFN-stimulated genes; however, some IFN-stimulated genes, including CXCL9, CXCL10, and CXCL11, and the IL-15α-receptor were not upregulated; (ii) $CD4^+$ and $CD8^+$ T-cells showed a cluster similar to that observed in thymocytes; (iii) more genes were differentially regulated in $CD8^+$ T-cells than in $CD4^+$ T-cells, including a cluster of genes downregulated exclusively in $CD8^+$ T-cells. According to their analyses, it appears that HIV-1 infection induces a persistent T-cell transcriptional profile, early in infection, characterized by a dramatic but potentially aberrant IFN response and a profile suggesting an active thymic output. They hypothesize that direct examination of $CD4^+$ and $CD8^+$ T-cells would yield further insights into why the majority of infected individuals are unable to control viral replication, and to identify why $CD4^+$ and not $CD8^+$ T-cells undergo progressive depletion. Overall, the selection of patients in this study, including that of LTNP, is too small to derive definitive and statistically meaningful conclusions. Thus, although this work provides important insights at the level of $CD4^+$ and $CD8^+$ T-cell transcriptome, more work is needed to address succinctly the clear differences between diverse rates of HIV disease, based on large cohorts of elite controllers as opposed to nonprogressors and progressors. The most up-to-date literature and a snapshot of all microarray data on human gene expression, in relation to HIV completed between 2000-2006, is reviewed by Giri, et al.[105].

HLA type and its influence on nonprogression

The identification of genetic differences between diverse individuals in host factors provides a means for a clear understanding of the genetic basis and the natural history of HIV disease. Although viral factors in concomitance with host-genetic factors may contribute to some cases of nonprogressing or slowly progressing HIV disease, they do not account for all cases of prolonged disease-free survival. The existence of such patients, who display some form of immunologic control over the HIV pathogen, provides the rationale for concerted efforts to better understand the host immune response in combating HIV infection[106,107]. Although a variety of host factors have been examined, a significant correlation has been observed among HLA genes. Several studies suggest that specific alleles of the HLA loci are associated with different rates of progression[108,109] and varying susceptibility to HIV infection[110]. Heterozygosity at all HLA class I loci appears to be protective against HIV infection, while class I alleles B35 and Cω4 have been consistently associated with accelerated progression to AIDS disease[109,111]. In a cohort of an HIV-infected Caucasian population, long-term nonprogression in 28-40% of nonprogressors was ascribed to heterozygosity at all HLA class I loci, the absence of alleles B35 and Cω4, or both[112]. The alleles B57 and B27 most consistently have been associated with long-term nonprogression. Homozygosity for HLA-Bω4 was also found to correlate with long-term nonprogression of HIV disease[113]. In addition, Migueles, et al. have also demonstrated a dramatic association between the HLA B*5701 class I allele, which is overrepresented in HIV-infected nonprogressors and nonprogressive HIV infection.

Eighty-five percent (11/13) of LTNP (normal CD4 counts, < 50 copies/ml of plasma) contained this allele, as opposed to only 11% observed in progressors[114,115]. These findings indicate that within this phenotypically and genotypically distinct cohort, a host immune factor is highly associated with restriction of virus replication and nonprogressive disease. Further characterization of qualitative differences in virus-specific responses that distinguish HLA B*57 LTNP from progressors may ultimately define mechanisms for effective immune-mediated restriction of virus replication. Furthermore, though the studies by Migueles, et al. have shown the biggest association occurring between B27 and B57 and long-term nonprogression, more robust associations with long-term nonprogression were observed with B14 and C8 alleles[116].

Both HLA-B*57 and B*58 are members of a rare HLA supertype family B58s. Rare HLA alleles are associated with low viral load related to CTL adaptation of the incoming virus[117]. Second, it has been proposed that the HLA-B*57 viral escape variants may be severely attenuated because of structural constraints[108,118], indeed resulting in a low viral load. Further, Kiepiela, et al.[119] have shown that variation in viral set-point, in absolute CD4 count and, by inference, in rate of disease progression, in the cohort is strongly associated with particularly HLA-B but not HLA-A allele expression. Also, greater selection pressure is imposed on HIV-1 by HLA-B alleles than by HLA-A. These data confirm that the principal focus of HIV-specific activity is at the HLA-B locus. It has been observed that B alleles evolve faster than A alleles and the HLA-B gene frequencies in the population are influenced by HIV disease. This dominant involvement of HLA-B in influencing HIV disease outcome may provide direction to HIV research and also to HIV vaccine design.

Recently, with the help of whole genome association strategy in one of the most elegant studies by a multinational team led by Fellay, et al.[120], based on 30,000 HIV patients and the selection of 486 patients with the most well-documented clinical time-points and viral loads, has unambiguously confirmed the protective association of HLA-B*5701 in HIV disease. In this study, the full genome scans, which can detect variations at 550,000 specific points, showed three distinct genetic variations, which strongly predicted low plasma viral loads consistent with a slower or nonprogressive HIV disease. These analyses identified two independently acting groups of polymorphisms, associated with *HLA* loci *B* and *C*, that are estimated to explain 9.6 and 6.5% of the total variation in HIV-1 set point, respectively, and can thus be considered as major genetic determinants of viral set point. One polymorphism is located in the *HLA* complex *P5* (*HCP5*) gene. The *HCP5* gene is located 100 kb centromeric from *HLA-B* on chromosome 6, and the associated variant is known to be in high linkage disequilibrium with the *HLA* allele *B*5701*[121]. This allele itself has the strongest-described protective impact on HIV-1 disease progression[114] and has been associated with low VL[122]. Interestingly, *HCP5* also happens to be a genetic fossil of an endogenous retroviral element, which has made its way into the human genome. The *HCP5* variation frequently occurs in conjunction with a particular version of an immune system gene called *HLA-B*. In light of strong functional data supporting a role for *HLA-B*5701* in restricting HIV-1, Fellay, et al. hypothesize that the association observed in their study is due to the effect of *HLA-B*5701*, reflected in its tagging a single nucleotide polymorphism within *HCP5*[121]. They emphasize, however, that genetics allows no resolution of whether the effect is exclusively due to *B*5701* or if *HCP5* variation also contributes to the control of HIV *in vivo*.

The second significant polymorphism identified is located in the 5' region of the *HLA-C* gene. Previously this gene has not been implicated in HIV control. This newly defined protective allele leads to a lower VL and is associated with higher expression of the *HLA-C* gene. This strong and independent association with *HLA-C* expression levels suggests that genetic control of expression levels of a classical *HLA* gene influences HIV control *in vivo*. Overall, these analyses have identified at least two mechanisms not known previously in the context of HIV control: *HLA-C*, which has been suspected but never confirmed to contribute to HIV-1 control, and an RNA polymerase subunit that substantially changes the time course of HIV progression. Fellay, et al.[120] also suggest the possibility that a human endogenous retrovirus-derived gene may contribute to the viral control attributed to the *HLA-B*5701* allele. Their findings not only validate but also emphasize the central role of the major histocompatibility complex region in HIV-1 restriction and the utility of high-density genotyping in HLA analysis.

Conclusions

The occurrence of adverse effects and the emergence of drug resistance associated with antiretroviral drug treatment, over time, are some of the major obstacles to long-term control of HIV infection and durability of sustained suppression of HIV *in vivo*. Further, since the discovery of the AIDS virus in 1983, though we have taken great leaps in understanding HIV, we have failed to deliver any effective anti-HIV vaccine. Therefore, new treatment strategies for HIV-1 infection are urgently needed. More than two decades have passed since the discovery of HIV and

there are at least 1% of HIV-positive, therapy naive individuals still living durably with HIV asymptomatically. Such are the individuals, who through natural control mechanisms can keep HIV at bay and have maintained a strong immune system, undetectable viremia and high $CD4^+$ and $CD8^+$ T-cell counts throughout the course of infection. For any treatment or vaccine strategies, a clear understanding of the natural control mechanisms of HIV *in vivo* is greatly needed. If we can understand the underlying mechanisms of host virus co-existence and the genetic/biological reasons for natural control of HIV over time in elite controllers, it may not only provide clues to vaccine and new therapeutic strategies, but may also shed light on some natural factors, which may find use as therapeutic vaccines in conjunction with currently prescribed therapies. While researchers are excited about the possibilities, there is skepticism surrounding the elite controllers, and many even realize prematurely that work on this subset of HIV individuals might not lead to any breakthroughs. In part, this pessimism can be attributed to the fact that it is unclear whether the resistance to immunologic damage by HIV in this group of nonprogressive individuals will last indefinitely, or whether it merely represents the extreme end of Gaussian distribution and eventually these individuals will progress to AIDS[18]. Nonetheless, whether elite controllers are a myth or a reality needs clarification because we are beginning to see clear evidence in favor of such a subgroup of HIV patients. A combined, multinational team effort is needed to address the underlying reasons for natural control of HIV in this subset of HIV-positive individuals. A bright future for research lies ahead and cutting-edge technologies of sequencing, full genome expression, and proteomic techniques such as DIGE and LC-Maldi, etc. may bring about new biological and structural knowledge of immune components that may revolutionize HIV research and change the way we look at HIV and its interaction with the human host in general.

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