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Elite controllers and lessons learned for HIV-1 cure

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Following the success of HIV-1 antiviral treatment that maintains undetectable levels of viral replication and lack of clinical progression, the design of an HIV-1 cure for patients became the next objective. The success of the treated individuals together with the identification of subjects that spontaneously control the clinical progression for long periods, such as long-term non-progressors (LTNPs) and particularly LTNP Elite Controllers (LTNP EC) have shed hope for the feasibility of a potential cure. Although a successful cure has not been attained yet, these patients have provided critical information on the mechanisms involved in the clinical control such as host genetic factors, as well as strong immune responses against the virus. Less attention has been paid to virological factors, particularly the association of the genetic variability and the control of viral infection. Considering all these studies, it has become clear that a combination of several host, immune and viral factors is needed to attain control of the viral replication control and the non-progressor clinical phenotype. Because this control can be reached through different combinations of factors, this group of individuals is not homogenous. As HIV-1 cure has been shown to be extremely difficult to achieve, a more feasible objective is the functional cure of the viral infection. After the analysis of multiple studies on the mechanisms of control in LTNP EC, we found subjects with various host protective factors and prolonged viral control. These subjects present a complete lack of evolution after more than 20–30 years of infection, stable levels of CD4+ cells (>400–500 cells/μl), a strong immune response, and no signs of clinical progression. We propose that individuals with these characteristics could have attained a functional cure of the HIV-1 infection.

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Introduction

The application of new and more potent antiretrovirals and novel drug combinations has permitted the control of the HIV-1 epidemic in the developed world. The mixture of drugs in combination antiretroviral therapy (cART) attains, in the treated patients, durable suppression of HIV-1 viremia. The control of viral production in cART patients led to the proposal for the potential cure of the HIV-1 infection [1]. Such cure, also named sterilizing cure, would imply the permanent control of viral replication and the complete elimination of the virus from the body. The elimination has been shown to be extremely difficult because viral reservoirs persist even under successful antiretroviral treatment. These remaining viral population in the reservoir constitute the principal burden for an effective HIV-1 cure, as they are the origin of a rapid rebound in viremia when treatment fails or is discontinued. Several strategies have been followed for the elimination of the HIV-1 reservoir without significant success. The majority of these strategies have focused on the reduction of the size of the viral cellular reservoir by the use of latency reactivating agents (LRA), like histone deacetylase inhibitors, protein kinase C agonists or NF-κB inductors, as single agents or in combinations [2,3]. However, at present these treatments have not produced a significant decrease in the reservoir [4].

The sterilizing cure has only been reported in two individuals, the Berlin and the London patients [5,6]. These cases, however, have followed extremely difficult approaches that are not feasible for the majority of the infected individuals. A less stringent objective, known as functional cure, consists in the permanent suppression of HIV-1 viral replication, in the absence of antiviral therapy, but without the need of viral eradication [7]. Recently, in a study with monkeys, the combination of two monoclonal antibodies (mAbs) delivered by adeno-associated virus vectors has resulted in the 'functional cure' of one the monkeys [8]. A situation resembling the functional cure is found among a minor group of HIV-1 patients, designated long-term non-progressors (LTNP) that control the viral infection and the clinical progression spontaneously for long periods. Within LTNPs, there is a subgroup called LTNP elite controllers (LTNP EC) that on top of this control maintain undetectable viral loads throughout infection [9]. These individuals have been extensively used for the investigation of the mechanisms involved in the control of the infection and they are considered a model for a functional cure of HIV-1 infection [10].

Definitions and characteristics of the LTNPS and LTNP EC patients

The studies performed with HIV-1 non-progressor (NP) patients have faced the problem relating to the disparity of the names and criteria used by the different groups in its definitions. These patients have been classified as NP patients, long-term HIV-1 positive (HLP), (LTNPs), or long-term survivors (LTS). Definitions were based on clinical and/or diagnostic criteria and years of follow-up. With the application of the viral load quantification (VL) in the patient's follow-up, distinct designations were again established such as elite suppressors (ES), HIV-1 controllers (HIC) or elite controllers (EC). For the definition of the LTNP EC individuals we propose: subjects who, in the absence of AIDS-related conditions and antiretroviral treatment, show HIV-1 infection for more than 10 years, stable CD4+ lymphocyte counts (>500 cells/μl) and undetectable viral loads (<50 cp/ml) [9].

Multiple works have been undertaken for the investigation of the causes of the viral infection control and the lack of clinical progression. The biomarkers involved in the clinical non-progressor phenotype are related to three main areas, those related to the host, those to the immunological response and those to viral factors.

Host factors

The first factors that were associated to the viral control in LTNPs were certain HLA alleles particularly the HLA B57*, B58* and B27* as well as other genotypes of the CCR5, CCR2, SDF1 and the KIR3DS1 genes because of their over representation in the NP patients. The multiple HLA alleles known to be associated with resistance to infection or delayed progression to AIDS have been recently reviewed [11]. Genome wide association studies detected new polymorphisms like the rs 9039999 SNP in the HLA C promoter and they further confirmed the above HLA alleles [12].

A combination of HLA-B57* and IFNL4 polymorphisms has been found to be associated with control of disease progression in controllers [3]. The important role for HIV control of the class I HLA peptide presentation was also described [12]. However, the contribution of these host markers to differences in the viral load is limited, and established at 30% in combination with viral factors [14]. Recently it has been suggested that peak viremia during the acute phase is likely lower in NP individuals than that in typical progressors because viral control by a favorable host response is active during the earliest stages of the infection [15].

One of the most significant host factors associated with viral control is the 32 bp deletion in the CCR5 gene (Δ32-CCR5 genotype). This genotype was associated with the LTNP phenotype because of a significantly higher proportion of heterozygous for the deletion in LTNPs, as confirmed in many cohorts. Other host restriction factors such as APOBEC3G, SAMHD1, Tetherin, and TRIM5α contribute to viral control but they also induce cellular resistance by innate and adaptive immune responses [16]. Also new restriction factors have been identified [17].

Factors related to the immune response

Several studies demonstrated that viral control in LTNPs is strongly correlated with the cellular response because of a tight association between Gag-specific cytotoxic CD4+ and CD8+ T lymphocyte responses, with a high polyfunctionality and viral control [18,19]. Moreover, CD8+ T cells from HIV-1 controllers exhibit a potent capacity to suppress viral infection '*ex vivo*' [19]. Also the combination of IL-18 and IP-10 plasma levels with activated CD8+ cells have been suggested as potential biomarkers of EC [20] as well as pro-inflammatory cytokines [19]. The CD8 (+) T cells restricted by the protective HLA alleles are not suppressed by Treg cells, in contrast with non-protective alleles [21]. The suppression of viral replication in EC has been also associated with host factors related to innate immunity, the humoral immune response, and the cellular immune response [22].

Antibody-dependent cellular cytotoxicity against envelope and vpu proteins, which is mediated largely by natural killer cells is associated with EC [23]. There are reports suggesting that the maintenance of memory B cells and a broad neutralizing capacity may contribute to the natural control of HIV-1 infection [24] particularly in ECs with HLA-B*57 allele [24]. Humoral responses and, in particular, neutralizing antibodies have been found in LTNPs [25,26]. In a study with monkeys, the combination of two or three potent neutralizing mAbs has resulted in the 'functional cure' of monkeys [8]. It has also been described that strong HIV-specific immune responses with low levels of inflammation are significant features of HIV controllers [27].

Virological factors

LTNP EC individuals display very low levels of virus, nucleic acids and proteins [28]. There is an ongoing debate about the characteristics of the viruses prevailing in LTNPs patients.

Defective viruses

In general, viruses from NP show low fitness and low replication capacity, or are defective in some of the viral proteins. The first characterization of defective viruses in non-progressor patients was in the Australian blood donor cohort which presented viruses with important deletions in *nef* gene [29]. In our group, we described an association between the lack of viral replication in LTNPs and the major presence of large genomic deletions in different genomic regions [30]. Defective provirus are the predominant forms in infected individuals as quantified by new comparative genomic assays [31].

Deleterious or attenuated viruses

Several studies established the low viral replication capacity of viruses from LTS, LTNP, and EC. The causes of such low replication capacity or viral fitness of these viruses were investigated and, in some cases, explained by the presence of CTL escape mutations in Gag proteins, some of which confer low fitness to the virus [32]. In addition, recombinant viruses with reverse transcriptase-integrase sequences from HIV-1 EC showed a reduced replication capacity [33].

Other reports focused on the phenotypic role of the viral envelope. Lassen *et al.* characterized the viral envelopes from a group of ES showing a significant decreased entry efficiency and a slow kinetic of the binding which contributed to the clinical ES phenotype [34]. Also an envelope with an unusual length in the V1 which conferred escape from monoclonal antibodies of the V3-glycan class was found in an elite controller [35]. In a study from our group, we analyzed the viral characteristics of the envelopes from a cluster of LTNP EC individuals with lack of clinical progression for more than 20 years. We established that these envelopes had a very low affinity for the CD4 molecule and a low transfer activity. These characteristics produced a very low cellular signaling within the host cell. All these viral properties could explain the low transmissibility and replication capacity of these viruses and the patients LTNP EC phenotype [36].

In contrast, Blankson *et al.* isolated viruses from elite suppressors (ES) with replicating characteristics similar to those of progressor patients [37]. This work established that the virus was not responsible for the non-progressor phenotype in ES. However, this result cannot be generalized because the virus was isolated in only four of the 10 patients studied. The replicative capacity of viruses from ES was confirmed in an '*ex vivo*' humanized mouse model also supporting the role of host factors in the viral control [38].

Despite the clonal expansion of replication-competent viruses long-term remission has been described in an HLA B57* EC [39]. However if individuals are infected with replicative viruses, these viruses could become deleterious because of the pressure of the host and immune system [10]. In summary, after reviewing all the virological studies, we can conclude that many LTNP EC show either no replicating virus, deleterious viruses or viruses with important defects. These alterations in the viruses result in low fitness and low replication capacity [40] with an important effect on the LTNP EC phenotype. We propose that these deleterious or attenuated viruses are a necessary component in the combinations of factors required for the control in LTNP EC.

Combination of factors

From the investigations carried out in LTNP EC many important conclusions can be derived. The control of viral replication in LTNP EC has been associated, in multiple studies, with different markers. Combined genotypes of CCR5, CCR2, SDF1, HLA genes and IFNL4 polymorphisms can predict the long-term non-progressor status in HIV-1 infected individuals [13]. In a systematic work from our group carried out with HIV-1 patients with distinct progression rates, we disclosed that there was a progressive accumulation of viral and host factors related to viral control from the rapid progressors, to standard progressor, LTNP viremic and LTNP EC individuals. This work clearly demonstrated that a combination of several host and virologic factors was necessary to achieve the extreme LTNP EC phenotype [9].

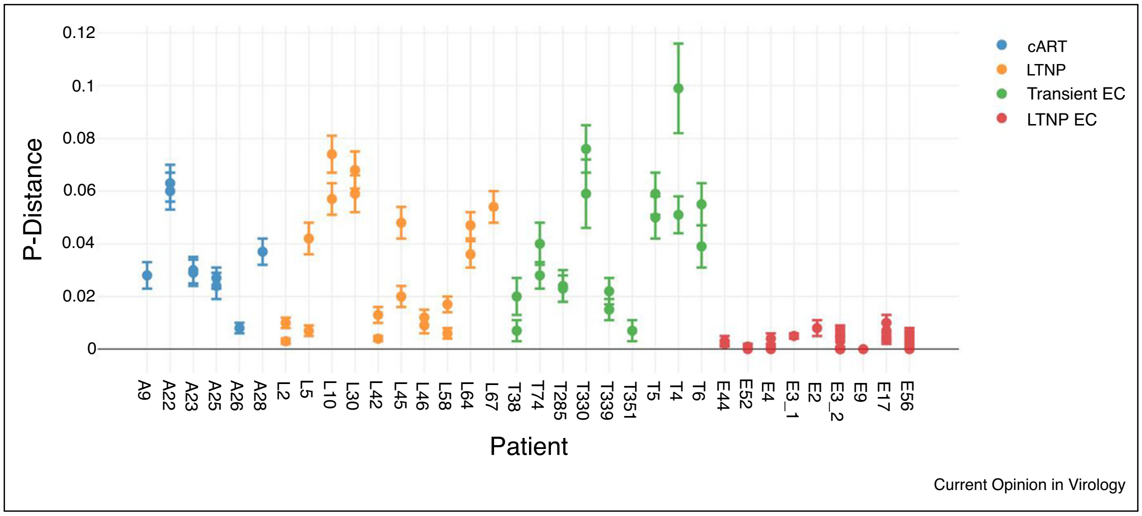
As a consequence of this multifactorial response and the different contribution of the distinct factors to the infection control, the group of LTNP EC is not homogenous. There are reports of LTNP EC that control viral replication without known host markers [41], or without protective HLA alleles [42]. Also, our group demonstrated that a combination of non-replicating viruses and a strong antiviral immune response explained the non-progressor phenotype in a super-infected HIV individual [43]. A general lesson from the LTNP EC studies is that the achievement of a complete control of the infection cannot be explained by a single factor, instead it requires a combination of multiple factors [14,44].

Genetic variability of viral populations in LTNP EC

HIV-1 replication is characterized by the generation of a high genetic diversity. This is due to the inaccuracy of the reverse transcriptase that lacks proofreading activity [45]. Thus, viral replication results in a diverse viral population best described as viral quasispecies [46]. Not only the genetic diversity of the HIV-1 populations, but also the size of the populations, will have consequences for viral fitness and pathogenesis. From genetic studies, it is known that small populations are prone to fitness losses because of the irreversible accumulation of deleterious mutations as previously described '*in vitro*', due to the operation of the Muller's ratchet [47]. LTNPs and LTNP EC show low or undetectable viral populations sizes [28] and, thus, are susceptible to the Muller effect.

In addition, the correlation between genetic variability and viral fitness has been established and was experimentally demonstrated with HIV-1 '*in vitro*' studies [44]. The association between viral diversity and pathogenesis was described in HIV-1 patients with different clinical progression [48]. In a work of our group with 14 LTNPs (CD4+>500, 10 years), individuals without a complete control of viral replication showed some degree viral evolution, whereas those with lower viral load showed an absence of viral evolution (see legend of Figure 1). Very different patterns of viral genetic diversity were found among HIV controllers [49]. In another study of

Figure 1



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Estimates of average evolutionary diversity over sequence pairs within individual and time point. The number of base differences per site (P-Distance) from averaging over all sequence pairs within each time point analyzed for every individual are shown. Standard error estimates are shown for each data point and were obtained by a bootstrap procedure (500 replicates). Values are colored according to the individual's clinical group. We included infected individuals under cART [50], LTNPs [51], and Transient 'Elite Controllers' [19] to compare to the viral populations from the LTNP 'Elite Controllers' [19]. Most of the individuals had multiple time points available.

our group with a cohort of EC controllers, individuals who showed a persistent control of viral replication displayed extremely low genetic diversity whereas EC with a transient control displayed a certain degree of viral diversity [19], thus supporting the role of viral diversity in viral evolution and pathogenesis.

Analysis of genetic variability in viruses from distinct groups of patients

To complement these studies on viral genetic variability, we compared the quasispecies heterogeneity in viral populations from different groups of individuals with a controlled infection such as patients under cART, LTNPs and the group of Elite Controllers with a transient or a permanent control [19]. We performed this analysis taking advantage of sequences deposited in the HIV Database (www.hiv.lanl.gov). Although both, LTNPs EC and cART treated patients maintain undetectable viral loads during many years of infection, genetic diversity among them is different. As shown in Figure 1, LTNP EC patients displayed an extremely low genetic diversity (the lowest diversity of all the patients analyzed) and it was lower than in the cART patients. Moreover, the genetic diversity remained constant during the infection. In contrast, in patients where there is evidence of viral replication like viremic LTNPs and transient EC, the genetic diversity was higher, it varied widely among them, and more remarkably, it changed during the patients' follow-up.

Some of the LTNP EC patients analyzed were subjects infected for more than 25 years with no signs of clinical progression [9,36]. In this group of individuals, even if residual replicating viruses still persist, we hypothesize that the low genetic diversity and low viral population size will make very improbable or even impossible the fitness recovery. This hypothesis is based on our previous '*in vitro*' study on Muller's ratchet, which establishes that quasispecies of HIV-1 with low diversity and low population size accumulate, during replication, harmful mutations that irreversibly lead to extinction [47].

The analysis of viral genetic diversity could be a very useful biomarker for the identification of individuals, mainly in the LTNP and LTNP EC, with a strong and prolonged control of viral replication and no evolution. The LTNP EC individuals with these characteristics are good candidates for a functional cure of the HIV-1 infection. Thus, the genetic variability could be a good prognostic marker of viral control and a surrogate for an HIV-1 functional cure.

A lesson that could be derived from these studies on genetic diversity is that if you attain a small viral population size and a low viral diversity, even with some replicating virus remaining in the individual, the virus will not be able to recover viral fitness.

Conclusions

The eradication of the HIV-1 virus from all the different body compartments is extremely difficult and thus far is not practicable as a general strategy for the control of the HIV-1 epidemic. A less ambitious objective, the functional cure of HIV-1 infection seems to be a more practical and feasible solution. As the functional cure has not yet been defined, we propose the following characterization: individuals with several protective host markers (e.g. HLA B27*, B57*/B58* and other polymorphism), prolonged viral control (>30 years) and no viral evolution, stable levels of CD4+ cells (>400–500 cells/μl) (because of strong immune responses) and without any sign of clinical progression. Perhaps there are individuals fulfilling these criteria, mostly within the LTNP EC cohort that could have already attained the functional cure of the HIV-1 infection.

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Conflict of interest statement

Nothing declared.

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