Lessons to be learned from natural control of HIV – future directions, therapeutic, and preventive implications

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Accumulating data generated from persons who naturally control HIV without the need for antiretroviral treatment has led to significant insights into the possible mechanisms of durable control of AIDS virus infection. At the center of this control is the HIV-specific CD8 T cell response, and the basis for this CD8-mediated control is gradually being revealed. Genome wide association studies coupled with HLA sequence data implicate the nature of the HLA-viral peptide interaction as the major genetic factor modulating durable control of HIV, but host genetic factors account for only around 20% of the variability in control. Other factors including specific functional characteristics of the TCR clonotypes generated *in vivo*, targeting of vulnerable regions of the virus that lead to fitness impairing mutations, immune exhaustion, and host restriction factors that limit HIV replication all have been shown to additionally contribute to control. Moreover, emerging data indicate that the CD8$^+$ T cell response may be critical for attempts to purge virus infected cells following activation of the latent reservoir, and thus lessons learned from elite controllers (ECs) are likely to impact the eradication agenda. On-going efforts are also needed to understand and address the role of immune activation in disease progression, as it becomes increasingly clear that durable immune control in ECs comes at a cost. Taken together, the research achievements in the attempt to unlock the mechanisms behind natural control of HIV will continue to be an important source of insights and ideas in the continuous search after an effective HIV vaccine, and for the attempts to achieve a sterilizing or functional cure in HIV positive patients with progressive infection.

Keywords: HIV, elite controllers, CD8$^+$ T cells, immune activation, HIV vaccine

INTRODUCTION

As the vigorous research of elite controllers (ECs) continue to unveil the various mechanisms behind durable and natural control of HIV, a complex image that includes different factors is exposed. Part of this milieu are components of the adaptive immune system, mainly CD8$^+$ and CD4$^+$ T cells, innate immune responses and cellular restriction factors, as well as specific characteristics of the infecting virus itself (reviewed in Deeks and Walker, 2007). CD8$^+$ T cells were repeatedly found as one of the major contributors to natural control of HIV. Genome wide associations studies consistently recognized the HLA peptide-binding grove (which binds to the CD8$^+$ T cell receptor, as well as to receptors on other cells of the immune system) as one of the central genetic associations with HIV disease outcome (Fellay et al., 2007; International HIV Controllers Study et al., 2010). Specific qualities of CD8$^+$ T cells from EC were recognized including their ability to inhibit HIV replication *ex vivo* (Sáez-Cirión et al., 2007), to deliver cytotoxic granules to HIV infected target cells (Migueles et al., 2008), to proliferate better (Migueles et al., 2002) and to exhibit multiple effector functions at the same time ("polyfunctionality") (Betts et al., 2006; Ndhlovu et al., 2012a). It was also demonstrated that CD8$^+$ T cells from EC tend to be more cross-reactive (recognizing virus variants and mutants) (Kosmrlj et al., 2010) and to preferentially target more conserved and vulnerable parts of the HIV

virus (where the virus pays a higher fitness cost in order to escape from the immune pressure exerted by cytotoxic T cells, CTL; Miura et al., 2009).

It has been shown that CD4$^+$ T cells from EC tend to be more polyfunctional, produce more IL-2, and proliferate better (Dyer et al., 2008; Ferre et al., 2010; Vingert et al., 2010). A few studies have also demonstrated that CD4$^+$ T cells from EC have reduced permissiveness to HIV infection (Graf et al., 2011; Sáez-Cirión et al., 2011). In that context, it was recently described that cell restriction factors might contribute to the lesser vulnerability of CD4$^+$ T cells of EC to HIV infection and replication (Chen et al., 2011). To that we should add the numerous reports that demonstrated important contributions of the innate immune system to elite control including natural killer (Lambotte et al., 2009; Vieillard et al., 2010) and dendritic cells (Huang et al., 2010; Barblu et al., 2012), as well as the contribution of special characteristics of the infecting virus itself (Miura et al., 2010).

Despite the impressive achievements in the field of EC research, our understanding of the mechanisms behind natural control of HIV is still substantially lacking, and the unknown is likely far greater than the known. In this review we examine these achievements in light of their potential contribution to future strategies for prevention and treatment, including vaccine design and attempts for a functional or sterilizing cure. In this way we

try to demonstrate that the research of EC goes beyond a pure academic exercise. We emphasize lessons learned from the findings achieved to date and their implications in planning the future steps in the research of this unusual group of patients.

LESSON I – FROM IMMUNOLOGY OF ELITE CONTROLLERS TO HIV VACCINE DESIGN

A major motivation behind the intensive study of EC is the intention to support the development of an effective HIV vaccine. If we closely examine the insights achieved by the study of EC during the past two decades we can see that several important immune correlates were added to our toolkit to help evaluate vaccine candidates, and some important principles learned can shed light on some of the failures and successes in the field of HIV vaccine research.

In Merck's phase IIb efficacy (STEP) trial, a replication-defective adenovirus construct (Ad5) containing *Gag*, *Pol*, and *Nef* genes was designed to induce T cell immune responses. The study was prematurely halted as it failed to demonstrate protection from acquisition of infection or to influence post infection viral load (Buchbinder et al., 2008). Indeed, early analysis suggested that the vaccine increased the risk of acquisition. A few principles learned in EC can be demonstrated when examining this important clinical trial. The failure of the vaccine was a surprise, mainly because of positive indications of immunogenicity in earlier phases of the vaccine or similar approaches in non-human primates (Shiver et al., 2002; Priddy et al., 2008), and the understanding that T cells play a central role in natural control of HIV, as demonstrated earlier through $CD8^+$ T cell depletion experiments in an animal model of AIDS virus infection (Jin et al., 1999; Schmitz et al., 1999), and by the strong association between certain HLA class I alleles and disease outcome (Fellay et al., 2007; International HIV Controllers Study et al., 2010). The major correlate of protection used to evaluate cell-mediated immune responses in the STEP study was interferon-γ enzyme-linked immunosorbent spot assay (ELISPOT). Such responses were not expected to prevent infection but rather to reduce steady state viremia, but despite induction of these responses, there was no enhanced control of set point viremia following infection (Buchbinder et al., 2008). Interferon-γ-secreting HIV-specific T cells were detected by ELISPOT in the majority of vaccinees, with similar proportion of responders both in those that eventually became infected and those who remained HIV negative (McElrath et al., 2008). Like this vaccine trial, there is no correlation between cellular immune responses measured by ELISPOT assays and clinical outcome (mainly viral load) in several studies looking at EC, yet the clear association of certain HLA alleles with control suggests that $CD8^+$ T cell responses play a role (Betts et al., 2001; Addo et al., 2003). The responses measured in the vaccinees in the STEP trial were significantly narrower and of lower magnitude in comparison to responses in EC (McElrath et al., 2008) or in monkeys achieving virologic control after vaccination with adenovirus vector-based SIV vaccine (Barouch et al., 2012). But in that context it should be emphasized that the magnitude and breadth of responses in ELISPOT assays were shown to be a poor predictor of HIV control (Betts et al., 2001), and in fact, it was demonstrated that $CD8^+$ T cell responses in EC are usually narrower and more focused on specific epitopes, especially in *Gag* (Migueles et al., 2000). In the STEP trial, on the other hand, $CD8^+$

T cell responses were more commonly directed toward *Pol* and *Nef* than toward *Gag* (McElrath et al., 2008). Another major difference between vaccinees in the STEP trial and subjects naturally controlling HIV lies in the way $CD8^+$ T cells exhibit their effector functions. While several studies have shown that an effective T cell response in EC is usually polyfunctional (i.e., $CD8^+$ T cells are able to produce multiple cytokines simultaneously) (Betts et al., 2006), in the STEP trial most vaccine-induced $CD8^+$ T cells produced interferon γ alone or in combination with TNFα only (McElrath et al., 2008).

It's also worth noticing that in the STEP trial only 41% of the vaccinees developed HIV-specific $CD4^+$ T cells that have the potential to support and maintain long-term antiviral $CD8^+$ T cell memory, and only 31% mounted both $CD4^+$ and $CD8^+$ HIV-specific T cells (McElrath et al., 2008). The ability to induce and maintain effective memory is probably one of the central characteristics expected from an effective vaccine. This principle is also important for natural control of HIV, and favorable characteristics of T cell memory have been demonstrated in EC (Ndhlovu et al., 2012b). It has been shown that EC show both effective and functional memory responses, and that central-memory (T_{CM}) and effector memory (T_{EM}) $CD4^+$ T cells in EC are less susceptible to apoptosis and persist longer after repeated stimulations as compared to aviremic HIV infected subjects treated with anti-retroviral treatment (van Grevenynghe et al., 2008). Furthermore, it has been recently shown that T_{EM} are the most effective $CD8^+$ T cell sub-population at suppressing viral replication in EC (Buckheit et al., 2012). In contrast to states of high antigen load (as is expected in uncontrolled HIV infection), it has been shown that memory cells in states of lower antigen load are able to persist longer, show lower degree of senescence, higher proliferative capacity, and therefore better functional properties (West et al., 2011). Therefore, EC are likely to be an important indicator for the desired way of maintaining memory $CD4^+$ and $CD8^+$ T cells.

The relevance of these findings to vaccine research was demonstrated in a recent proof-of-concept study in which researchers have used an SIV protein-encoding vector based on rhesus cytomegalovirus (Hansen et al., 2009, 2011). By using a persistent vector that leads to a controlled, continuous level of antigen expression, robust SIV-specific $CD4^+$ and $CD8^+$ T_{EM} cell responses were primed and maintained. This approach led to impressive protection from acquisition (significantly higher number of challenges needed to cause infection), and importantly to control of post infection viremia in the monkeys, a state that may be similar to elite control or functional cure in humans. The effectiveness of this new approach (in NHP at this stage) renders great hope in the field of HIV vaccine research (Walker et al., 2011). In contrast to older strategies (as that used in the STEP trial) when a non-persistent vector is used and T_{CM} is the predominant memory population elicited, this new strategy leads to development and maintenance of a potent T_{EM} population, thus a faster and more effective systemic and mucosal T cell responses develop. Our own data show that the HIV-specific T_{EM} population in EC predominates and is less prone to apoptosis/deletion as compared to CP (unpublished data).

Although extrapolation of immune correlates of protection in chronic HIV infection to predict protection in vaccine trials might

be misleading, the field of HIV vaccine development desperately needs better-defined immune measures that are able to predict vaccine efficacy. The study of ECs, by detailed dissection of the components that lead to natural control, added several such correlates, and it would be reasonable to assume that some of them would be considered in future clinical trials. This includes the ability of CD8$^+$ T cells to directly inhibit HIV replication *ex vivo*, their ability to proliferate, and to exhibit polyfunctional effector profile, as well as the composition of the memory subsets induced.

LESSON II – ELITE CONTROLLERS, FUNCTIONAL CURE, AND HIV ERADICATION

The most prominent characteristic of EC, complete suppression of the viremia to undetectable levels, can be easily achieved by combination antiretroviral treatment. But some unique and desirable features in EC are absent in non-controllers treated with HAART. Obviously, in contrast to EC, the suppression of the viremia in HAART treated patients requires lifelong medications that are both expensive, and carry the risk for toxicity. Until recently, treatment interruptions universally resulted in viral rebound, and lack of adherence carries the risk for emergence of resistant virus that further complicates the therapeutic regimen. Life expectancy has never been compared between a cohort of untreated EC and non-controllers treated with antiretrovirals, but it has been demonstrated that subjects naturally controlling HIV have smaller HIV reservoirs compared to progressors treated with HAART (Graf et al., 2011; Buzon et al., 2012), and that recovering virus from EC seems to be more difficult as compared to HAART treated patients (Julg et al., 2010). Therefore, it appears as EC are an excellent model of functional cure, a state in which the virus is maximally suppressed, although not cleared, and the integrity of the immune system is maintained relatively intact by the patient's own defense mechanisms and without the need for life long medications.

Elite controllers inspired researchers to try to induce a similar state of control in infected subjects that do not control the infection spontaneously, and research is on its way in that direction. The most encouraging attempt in that direction was achieved in patients treated with antiretrovirals shortly after infection. In a recent report from France 12 patients out of 75 (16%) who started combination antiretroviral treatment within 10 weeks after HIV infection kept their viral load <50 copies/ml for more than 6 years after discontinuing the antiretroviral treatment (Hocqueloux et al., 2010; Bacchus et al., 2012). The prevalence of elite control in that intervention group was more than 30-fold higher compared to a cohort of non-selected HIV infected individuals (in which the frequency of EC is usually <0.5%). In contrast to EC, the acutely treated cohort was not enriched with protective HLA alleles like HLA-B57 or B27, but rather with HLA-B35, which is associated with rapid disease progression (Carrington et al., 1999), but the reservoir (HIV DNA) in treated patients becoming controllers had similar characteristics compared to EC in terms of magnitude and distribution.

The principle of early treatment was also employed in an interesting case of congenital HIV infection, in which immediate treatment after birth with a full dose antiretroviral regimen led to sustained suppression of viral replication despite prolonged treatment discontinuation (Persaud et al., 2013). These cases of

early treatment differ in the approach taken compared to other early intervention studies in both children and adults, in which pulse therapy was largely used, and duration of treatment was much briefer in these patients who did not control after treatment discontinuation (Kaufmann et al., 2004; Fortuny et al., 2011).

It would be important to reveal the mechanisms involved in inducing a state of viral control in patients not harboring protective HLA alleles treated early during the course of acute infection. In a study by our group interesting insights came up from close examination of the transition from acute to chronic controlled infection (Miura et al., 2010). Looking at a cohort of acutely infected subjects not enriched with protective HLA alleles that eventually became HIV controllers significantly higher prevalence of drug resistance or CTL escape mutations associated with impaired viral fitness was found in controllers compared to non-controllers. This work implies that the characteristics of the transmitted virus itself can dictate the fate of the infection natural course. Further examination of the mechanisms leading to elite control after acute infection would broaden our understanding of the way patients become ECs and ways to intervene and induce such a state.

Lessons learned from EC are also influencing cure strategies involving gene therapy. The class I HLA alleles B57 and B27 are the most recognized genetic elements associated with HIV control, and recently several studies have demonstrated that HLA-B27 and B57 restricted TCR clonotypes from EC have superior ability to suppress HIV-1 replication *in vitro* (Chen et al., 2012), greater cross-reactivity to epitope variants (Chen et al., 2012; Ladell et al., 2013) and enhanced loading and delivery of perforin in comparison to HLA-B27 and B57 restricted TCR clonotypes form non-controllers (Chen et al., 2012). Researchers are now examining the possibility to modulate the TCR repertoire of patients not carrying protective HLAs, or having immunodominant non-effective TCR clonotypes by TCR gene transfer (Varela-Rohena et al., 2008; Barsov et al., 2011; Hofmann et al., 2011). By doing that, the purpose is to provide CD8$^+$ T cells clones with all the superior characteristics recognized in patients controlling HIV. This idea was proven promising in an animal model at this stage, but active research is still on its way. While adoptive transfer of *ex vivo*-expanded autologous or allogeneic HIV-1-specific CTLs into HIV-1-infected individuals had minimal effect on HIV-1 viral load (Lieberman et al., 1997; Tan et al., 1999; Bolton et al., 2010), as the immunodominant CTL clones in patients with progressive infection are usually exhausted and more susceptible to apoptosis, transduction of primary CD8$^+$ T cells with TCR genes from *Gag* specific clone harboring potent inhibition capacity led to an impressive *in vivo* inhibition of HIV in a mouse model (Joseph et al., 2008). This exciting idea is awaiting further evaluation in NHP models and in humans.

The use of genetic engineering in the field of HIV cure research has received inspiration from some other findings in EC and by examining mechanisms involved in viral entry. It is well established that a 32 base pair deletion in the HIV co-receptor C–C chemokine receptor 5 (CCR5) gene confers profound resistance to infection by R5 tropic viruses, and indeed, it has been shown that Δ32 heterozygosity is more prevalent among EC or is associated with lower viral load (Magierowska et al., 1999; International HIV Controllers

Study et al., 2010). This important notion not only served the field of antiretroviral therapy development (Gulick et al., 2008), leading to a pharmacologic intervention to block this receptor, but was also employed in an outstanding key work in which HIV cure was achieved by allogeneic bone marrow transplantation in HIV infected patient with acute leukemia. This patient, known as the "Berlin patient," received hematopoietic stem cell (HSC) graft from a donor homozygous for Δ32 (Hütter et al., 2009) and eventually achieved sustained undetectable viremia despite discontinuation of antiretroviral treatment for more than 3 years (Allers et al., 2011). The same idea is being examined in several studies in which researchers are trying to artificially induce a deletion in the co-receptor by genetic engineering using zinc finger nucleases (ZFN) (Holt et al., 2010). Doing so, researchers were able to disrupt the CCR5 gene in human HSCs and to engraft them into immunodeficient mice. Mice transplanted with ZFN-modified HSC, and later infected with HIV, underwent rapid selection for CCR5-negative cells, and showed significantly lower HIV-1 levels in both peripheral blood and gut mucosa, as well as restoration of normal levels of human $CD4^+$ T cell levels throughout their lymphoid tissues. Examination of this concept in humans is currently underway (Tebas et al., 2011; Maier et al., 2013).

Additional work is now taking place examining ways to promote HIV cure, and an international consortium was established specifically for that purpose (International AIDS Society Scientific Working Group on HIV Cure et al., 2012). No doubt that insights learned from the immunology and virology of EC will continue to provide major support to those efforts. The ideas under examination that are inspired by EC research include the efforts to induce strong and effective T cell responses by vaccination [an area that experienced failures and disappointments (Autran et al., 2008), but received some positive results recently utilizing dendritic cells based therapeutic vaccine (García et al., 2013)]. Efforts are also concentrated on attempts to attack and reduce the viral reservoir, to eliminate latently infected cells by activation (using IL-7, histone deacetylase inhibitors, prostratin, and others) in conjunction with induction of effective cytotoxic responses, in addition to the previously mentioned ideas. The possibility of identifying a way for patients to stop antiretroviral treatment and acquire a phenotype similar to ECs is a great challenge that might have significant clinical, epidemiological, and financial implications, but is not without risks due to persistent immune activation. Therefore, scientists and clinicians from different disciplines must continue and work together to take this important goal forward.

LESSON III – ELITE, BUT IMPERFECT CONTROL – THE ROLE OF IMMUNE ACTIVATION

Life expectancy in HIV infected individuals has improved dramatically since the introduction of highly active antiretroviral treatment (Bor et al., 2013), but studies show that it is still compromised compared to matched uninfected controls (Lohse et al., 2007; Nakagawa et al., 2012). Non-AIDS events among HIV infected individuals (including malignancies, cardiovascular, liver, renal, bone disease, and others) are increasingly recognized since HIV became a chronic condition (Guaraldi et al., 2011), and it is clear now that undetectable plasma viremia is not sufficient to prevent such complications. Excess morbidity and mortality in treatment-suppressed HIV infected individuals were attributed to multiple etiologies including toxicity of the antiretroviral treatment, higher rates of co-infections (like HCV and HBV), life style and risk behavior (smoking, drug, and alcohol use), and lower socioeconomic status. But even after adjustment for those factors, higher rates of comorbidities were found compared to uninfected controls, and there is a broad recognition today that a major cause of that is the deleterious effect of the continuous inflammation and immune activation described in these patients. Increased levels of T cell activation (CD38 and HLA-DR), innate immunity activation (CD16 and IL-6 produced by monocytes, IFN-α by dendritic cells, and others) as well as markers of microbial translocation (LPS and other microbial products) and impaired coagulation (D-dimer) were found in chronically infected patients, both treated and untreated, and were associated with increased morbidity (Hsue et al., 2012; Marks et al., 2013; Morse et al., 2013) and mortality (Cozzi-Lepri et al., 2011; Achhra et al., 2012).

In that context, EC serve as an important model to study immune activation. By eliminating confounding factors present in other patient populations (the drug toxicity in HAART treated patients with undetectable viral load, or the influence of the on-going viremia in chronic untreated patients) researchers were able to get a better understanding of the pathogenesis and harm caused by immune activation.

Elevated markers of immune activation and bacterial translocation were found in EC as compared to HAART treated patients (Hunt et al., 2008, 2011), and these abnormalities were associated with lower $CD4^+$ cell counts. Excess morbidity from non-AIDS conditions was also found in EC compared to HAART treated patients with undetectable viral load. In a recent work, the prevalence of coronary atherosclerosis (estimated by coronary CT) was unexpectedly higher among EC with sustained viral suppression (no history of viral "blips") compared to HIV negative individual or HIV infected patients treated with antiretroviral and maintaining undetectable viral load (Pereyra et al., 2012). The higher prevalence of coronary disease was associated with elevated markers of innate immune activation (sCD163, a marker of monocyte activation) as well as markers of $CD8^+$ T cells activation (CD38 and HLA-DR). Increased levels of carotid artery intima-media thickness (another manifestation of accelerated atherosclerosis) was also found in EC, and was associated with C reactive protein, a marker of inflammation (Hsue et al., 2009). It has been shown that EC and viremic controllers (those controlling the virus to levels below 2000 copies/ml) have high level of collagen depositions and fibrosis in the gut associated lymphoid tissues, a feature of immune activation and end-organ damage (Sanchez et al., 2013). Surprisingly, the degree of collagen deposition was higher in EC compared to HAART treated patients, uninfected individuals or to that found in the "Berlin patient," known to be cured of HIV. Some other manifestations of immune dysfunctions were demonstrated in EC. A non-negligible portion (about 10%) of EC progress to AIDS or show progressive loss of their CD4 despite the undetectable viremia (Hunt et al., 2008). Furthermore, in studies in which EC initiated HAART for various clinical indications, $CD4^+$ T cell gain was inferior compared to that achieved in non-controllers (Okulicz et al., 2010). These findings imply that despite the impressive control of the plasma viremia in EC, still a

significant level of immune activation/dysfunction is present and is associated with various forms of end-organ damage, and might compromise long-term prognosis.

In addition to the observations of increased immune activation, the study of EC helped to explore the various etiologies contributing to its pathogenesis. Like in studies looking at HAART treated patients with undetectable plasma viremia (Buzón et al., 2010), data collected in EC showed that continuous viral replication exists despite the undetectable plasma viremia by standard assays, and it might play a major role in the pathogenesis of chronic immune activation. Utilizing ultrasensitive assays it has been shown that low-level plasma viremia is common in EC, and indeed, 98% of EC had detectable low-level viremia at some point during prolonged follow-up (Hatano et al., 2009). Median plasma viral load of 2 copies/ml was found in EC (Pereyra et al., 2009), a level higher than that usually documented in HAART suppressed patients (Hatano et al., 2009). Cell associated RNA and persistent cell integrated DNA were also evident in EC, and replication competent virus was isolated from these patients (Blankson et al., 2007). Furthermore, some studies have demonstrated that viral evolution occurs in EC, a clear evidence that on-going replication is taking place (O'Connell et al., 2010). These findings imply that the immune system of EC is continuously exposed to low-level release of viral products, which might trigger the state of continuous immune activation and chronic inflammation.

Other factors involved in the pathogenesis of immune activation were described in EC. It is well established that HIV infection is associated with disruption of the gut mucosal barrier and persistent translocation of bacterial products, which in turn lead to systemic immune activation (Brenchley et al., 2006; Wallet et al., 2010). Like in other cohorts of chronically HIV infected individuals, increased markers of microbial translocation were found in EC (Hunt et al., 2008). In a study that compared EC, HIV negative individuals, and untreated HIV infected patients, LPS levels were significantly higher in EC compared to HIV negative controls, and similar to the levels in untreated patients. These findings were associated with higher levels of $CD8^+$ T cells activation (Hunt et al., 2008). Such abnormalities might explain the higher levels of fibrosis in the gut associated lymphoid tissue of EC, mentioned before (Sanchez et al., 2013).

To that we should add other conditions that might play a role in the pathogenesis of immune activation in EC, including co-infections with other persistent pathogens like HBV and HCV, the highly effective immune responses in EC that play an important role in controlling the infection but at the same time might facilitate the state of inflammation and immune activation, and other etiologies yet to be defined.

Vigorous research is taking place these days exploring ways to minimize or prevent immune activation and its harmful consequences. The ideas being examined include attempts to eliminate residual viral replication by treatment intensification

with antiretrovirals (Buzón et al., 2010; Massanella et al., 2013), interventions to improve gastrointestinal tract integrity (Klatt et al., 2013) and to eliminate other persistent co-infections, treatment with non-specific inhibitors of chronic inflammation like statins, anti-inflammatory agents, anti-cytokine and anti-chemokine agents, immunosuppression, and others (Rajasuriar et al., 2013). The study of EC gives important support in that direction as well, taking advantage of the unique properties of this group of patients. In a recent report, patients naturally controlling HIV (both viremic and ECs) were thoroughly examined after 24 weeks of prospective treatment with combination of three anti-retroviral drugs (Hatano et al., 2013). This intervention led to a significant decrease in plasma RNA by ultrasensitive assay, decrease in markers of T cell activation in blood and the gut, and decrease in the percentage of $PD-1^+$ $CD4^+$ and $CD8^+$ T cells. This work demonstrates that significant reduction in immune activation in controllers is possible by eliminating residual viral replication. It would be interesting to examine the influence of other treatment modalities in this group of patients and especially in patients that effectively control HIV but still progress to AIDS.

CONCLUDING REMARKS

The phenomenon of natural control of HIV inspired many researchers who take part in the efforts to develop new preventive and therapeutic modalities for HIV. Exploring the impressive balance between the host and the virus, several new ideas came out that might serve the field of development and evaluation of new vaccine candidates and other modalities of immunotherapy, the field of HIV treatment, and the efforts to eradicate the infection and to lead to HIV cure. The study of EC taught us few lessons about the complex network of components that contribute to the natural control of the virus and the different arms of the immune system involved. The markers of control identified in EC might serve as an important and more accurate correlates of protection in studies of vaccine candidates, and effective immune responses might be transferred by genetic engineering into subjects not naturally controlling HIV. The study of EC also provided new ideas and tools to the field of HIV cure research and to the attempts to achieve HIV eradication, and different ways to induce a state similar to EC are still being examined. The damage caused by chronic inflammation and immune activation is increasingly recognized as an important element compromising the prognosis of HIV infected individuals, and a model of HIV infected patients with undetectable viremia and no exposure to antiretroviral medications is an important tool to study the etiology and influence of these deleterious processes.

As the mechanisms behind natural control of HIV are only partially understood, much effort is still needed to be carried out, and research must continue in new directions and toward new ideas. No doubt the findings revealed by these efforts will have a major impact on the way we treat and prevent HIV in the future.

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