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Review

# Effector mechanisms in HIV-1 infected elite controllers: Highly active immune responses?

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ABSTRACT

Elite controllers (EC) are HIV-1 infected patients control viral replication to a level of <50 copies/ml without antiretroviral therapy. These patients are also known as elite suppressors, or HIV controllers, and they differ from traditional long-term non-progressors (LTNPs) who maintain stable CD4 counts and are asymptomatic without antiretroviral therapy. Recent studies suggest that many EC are infected with replication-competent virus. Thus it appears that host factors such as innate immunity, the humoral immune response, and the cellular immune response are involved in the suppression of viral replication in EC. This article will review the effector mechanisms that are thought to play a role in the remarkable control of viral replication seen in these patients.

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**Contents**



## 1. Introduction

Less than 1% of all untreated HIV-1 infected patients will maintain viral loads of <50 copies/ml (Lambotte et al., 2005; Grabar et al., 2009). These elite controllers (EC) potentially serve as models for effective HIV-1 vaccination. It is therefore crucial that we fully understand the virologic, genetic, and immunologic factors that contribute to elite suppression of viral replication in these patients. Fortunately, EC have been the subject of intense research over the past few years and this has lead to a better understanding of the mechanisms responsible for elite control of HIV-1 infection. It is important to note that while low CD4+ T cells have been noted in a few EC (Greenough et al., 1999; Madec et al., 2005; Hunt et al., 2008; Andrade et al., 2008; Sedaghat et al., in press), the majority of these patients have very low rates of CD4+ T cell decline (Sedaghat et al., in press), and disease progression (Sajadi et al., 2009) and differ from viremic long-term non-progressors (LTNPs) (Buchbinder and Vittinghoff, 1999), who eventually develop progressive HIV-1 disease (Lefrere et al., 1997; Westrop et al., 2009). This review will thus focus on patients who have undetectable viral loads rather than LTNPs or patients with low viral loads who are also sometimes referred to as controllers.

## 2. Virologic factors in EC

Some studies have suggested that LTNPs and EC are infected with defective virus (Alexander et al., 2000, 2002; Calugi et al.,

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2006; Deacon et al., 1995; Huang et al., 1998; Iversen et al., 1995; Kirchhoff et al., 1995; Mariani et al., 1996; Salvi et al., 1998; Michael et al., 1995; Yamada and Iwamoto, 2000; Wang et al., 2003) which could potentially explain their clinical status. While it is clear that infection with defective virus can lead to control of viral replication, there are now several lines of evidence to suggest that many EC are infected with pathogenic virus. One study sequenced full length provirus and plasma virus in more than 60 EC and found no evidence of large deletions that would affect virulence (Miura et al., 2008). While actual replication-competent virus was not examined, the data suggested that EC are infected with virulent isolates. Other studies have shown that stimulation of EC CD4+ T cells with mitogens resulted in the detection of Gag within cells (Migueles et al., 2003) or in culture supernatant (Lamine et al., 2007). These results suggested that viruses were capable of replicating to some extent and sequence analysis ruled out deletions in *vif* and *vpr* (Lamine et al., 2007). In a detailed study, replication-competent virus was isolated from 4 EC and full length genome sequencing and phenotypic analysis was performed on the isolates (Blankson et al., 2007). This analysis strongly suggested that the viruses isolated from the EC were fully pathogenic. Furthermore virologic breakthrough was seen in an EC after a year of control. The fact that the patient's viral load eventually increased by more than 2 logs is proof that he was not infected with a defective virus (Bailey et al., 2007). Another study documented superinfection of an EC with a second HIV-1 isolate (Rachinger et al., 2008) and showed that while this patient maintained relative control of viral replication (VL of 2200 copies/ml), two other patients who were infected with similar isolates had very high viral loads.

The most definitive evidence that some EC are infected with pathogenic virus comes from a case report where a patient who developed AIDS was shown to transmit virus to an EC (Bailey et al., 2008). While virus isolated from the EC was less fit than the virus isolated from the progressor, it was shown that this was most likely due to the presence of escape mutations the virus from the EC had developed in response to the strong HIV-specific response. Thus the host response, rather than the degree of virulence of the infecting virus, can determine the outcome of infection in EC in some instances and this host response can also have an impact on viral fitness.

This concept of the immune response affecting fitness may explain why plasma *gag* (Miura et al., 2009b) and *env* (Lassen et al., 2009) clones from EC are less fit than clones from patients with progressive disease. Plasma clones from EC differ significantly from proviral clones (Bailey et al., 2006a, 2006b), probably as the result of selective pressure exerted by the immune response. Thus the fitness of these clones may not be representative of the fitness of the virus the patients were originally infected with. Studies comparing viral clones from the two compartments are needed to address this issue.

## 3. The role of innate immunity

Studies have dissected multiple aspects of the host response in EC in effort to determine what is most responsible for controlling the replication of pathogenic virus. Several studies have shown normal replication of heterologous and autologus HIV-1 isolates in activated CD4+ T cells from ECS (Wang et al., 2002; Blankson et al., 2007; Lamine et al., 2007). Thus these cells are not inherently resistant to HIV-1 infection. Other studies have focused on the APOBECEG/F, enzymes that are capable of inactivating HIV-1 by G to A hypermutation (Goila-Gaur and Strebel, 2008). A study showing that all HIV-1 proviral *gag* clones amplified from an EC had several premature stop codons due to hypermutation suggested that this was potentially the mechanism of control of viral replication in some patients (Wang et al., 2003). However, another study showed there was no difference in the frequency of hypermutated proviral clones in a cohort of EC versus patients with progressive disease on HAART (Gandhi et al., 2008). Furthermore, while hypermutation was seen in 80% of proviral clones from an EC in this study, multiple viral clones amplified from the plasma of this patient were not hypermutated. In addition, the *vif* gene, which counteracts APOBEC 3 activity, appeared to be fully intact, and viral isolates could be easily grown in activated CD4+ T cells from this EC, suggesting that the high degree of APOBEC activity was not an absolute block to HIV-1 replication (Gandhi et al., 2008). Thus enhanced APOBEC activity does not appear to be the mechanism responsible for control of replication in most EC.

Toll like receptors are involved in the innate recognition of many microbes including HIV-1 via pathogen associated molecular patterns (PAMPs). Polymorphisms in these receptors have been shown to influence disease outcomes in different infectious diseases (Lasker and Nair, 2006). While polymorphisms in TLR9 has been shown to be associated with rapidly progressive HIV-1 infection (Bochud et al., 2007), there have been no studies linking TLR polymorphisms to elite control of HIV-1 infection. Interestingly HIV-1 endocytosis has been shown to activate plasmacytoid dendritic cells (pDCs) by TLRs (Beignon et al., 2005; Fonteneau et al., 2004) leading to the secretion of interferon-alpha and other inflammatory cytokines which may have antiviral properties. While the frequency of pDCs have been shown to decline with progressive HIV-1 infection, LTNPs have higher numbers of these cells than uninfected subjects suggesting that these cells may contribute to the control of HIV-1 replication (Soumelis et al., 2001).

Natural killer cells are involved in the innate control of many viruses. Several lines of evidence have suggested that these cells may also be playing a role in the natural control of HIV-1 infection. HIV-1 infection can lead to the downregulation of HLA-A and HLA-B proteins on the surface of infected cells (Cohen et al., 1999), a process that has been shown to trigger NK cell activation. However, HLA-C molecules, which are not downregulated by HIV infection (Cohen et al., 1999), may provide some protection from NK cells. Interestingly, in multiple studies (Fellay et al., 2007; Catano et al., 2008; Shrestha et al., 2009; van Manen et al., 2009), a single nucleotide polymorphism associated with the HLA-C promoter has been associated with low viral loads. This polymorphism was absent in 75% of patients in a cohort of African American EC (Han et al., 2008), but it is possible that when present, it may affect the interaction between HLA-C molecules and their ligands on NK cells in a manner that results in effective killing of HIV-1 infected cells.

Certain alleles of killer immunoglobulin receptors (KIRs) on NK cells are associated with slowly progressive disease. In a large epidemiologic study it was shown that patients who had both the KIR3DS1 allele and HLA class I alleles that encode an isoleucine at position 80 (HLA-Bw480I), had the slowest rates of progression to AIDS (Martin et al., 2002). It should be noted that HLA-Bw480I alleles include alleles such as HLA-B*57 which are by themselves associated with protection from progressive HIV disease (Kiepiela et al., 2004), but the presence of KIR3DS1 conferred additional survival benefit (Martin et al., 2007). Another large cohort study showed that the presence of HLA-Bw480I alleles and certain KIR3DL1 alleles also delayed the progression to AIDS (Martin et al., 2007). KIR3DS1 is an activating NK cell receptor whereas KIR3DL1 is an inhibitory receptor. While there is no direct evidence of an interaction between either of these receptors and HLA-molecules, the data could be potentially explained by either enhanced activity or diminished inhibition of NK cells that expressed KIR3DS1 or KIR3DL1 respectively upon interaction with infected cells that had downregulated HLA-Bw480I proteins.

There have been very few studies looking at the expression of KIRs and the HLA-C SNP in EC. In a cohort of 20 African American ES, 17 had HLA-Bw480I alleles but only 2 patients were found to be

KIR3DS1 positive and just one patient had both alleles (O'Connell et al., 2009). While this KIR allele is very rare in African Americans, the fact that it was not over-represented suggest that it is not essential for the elite control of HIV infection. The protective KIR3DL1 alleles were over-represented in patients with viral loads of <2000 copies/ml (Martin et al., 2007), but this finding was not confirmed in a cohort of EC when the effect of protective HLA alleles was taken into account (Jagannathan et al., 2009). Larger cohorts of EC need to be studied, but the preliminary data suggests that while these KIR alleles in conjunction with HLA-Bw480I alleles are clearly important in traditional LTNP, they may have less of a role in EC.

Barker et al. showed that the phenotype of NK cells from EC was very similar to that of NK cells from patients on suppressive HAART regimens; both groups had high levels of $CD56^{dim}CD16^{+}$ NK cells that are thought to have cytotoxic potential and low levels of the $CD56^{-}CD16^{+}$ dysfunctional NK cells that are seen in viremic patients. The authors concluded that NK cells were probably not responsible for the control of viral replication in EC (Barker et al., 2007). There has been just one functional study of NK cells in ES and it showed that these cells were significantly less effective than CD8+ T cells at inhibiting HIV-1 replication in vitro even when IL-2 was used to activate the NK cells (O'Connell et al., 2009). It is possible that even low level killing of infected cells during primary infection may lead to elite control of viral replication. It is also possible that these cells are not involved in the direct killing of HIV-infected cells in EC, and instead may modulate cytokine secretion through their interactions with pDCs, leading to more effective adaptive responses. Understanding the early events that occur in primary infection will probably be critical to the understanding the mechanisms involved in elite control of HIV-1 replication.

## 4. The role of humoral immunity

Neutralizing antibodies are thought to function by binding to envelope proteins thereby preventing the interaction with the cellular receptors that leads to viral entry. In HIV-1 infection, this is a particularly important step since once infection occurs, the latent form of the virus makes eradication by either the immune system or HAART essentially impossible. Thus the ultimate goal of an HIV-1 vaccine would be induction of an antibody response that is capable of effectively neutralizing multiple different strains of HIV-1 from different clades. Investigators have studied the role of neutralizing antibodies (Nab) in EC. Laeyendecker et al. (2008) showed that chronically infected ES had very low titers of HIV-specific antibody and thus could be mistaken for recently infected patients with the Vironositika-LS EIA assay. The avidity of these antibodies however was quite high and it was therefore possible that antibodies in these patients were still capable of strong neutralization activity. This has been investigated by several different groups. A study comparing EC to patients with low level viremia and chronic progressors (CP) found that EC had significantly lower titers of Nab to laboratory isolates as well as to pseudotype virus expressing Env from CP and patients with low levels of viremia (Pereyra et al., 2008). Doria-Rose et al. (2009) looked at the ability of sera from HIV-1 infected patients to neutralize a panel of 5 HIV-1 isolates from 3 different clades. They demonstrated that only 25% of EC had broadly cross-neutralizing antibodies compared to 41% of slow progressors and 42% of progressors. This confirmed data from prior studies where EC were found to be less likely to have broadly neutralizing antibodies than viremic patients with slowly progressive disease (Li et al., 2007).

These data suggest that EC are very different from slow progressors and traditional viremic LTNP. Lambotte et al. (2009) found that EC and CP had no difference in the titers of antibodies to different Env epitopes including the CD4 binding site. They also showed that Nab titers were lower in EC than in progressors, however when they looked at antibody dependant cell cytoxicity, they found that EC had significantly higher levels than progressors. Scheid et al. (2009) demonstrated that antibodies from EC with broadly neutralizing antibody activity were specific for multiple epitopes on Env, but there was no single monoclonal antibody from any individual that was capable of broad neutralization. Instead it appears that combined activity of different monoclonal antibodies provided the broad neutralizing activity. It should be noted that only 12% of EC in one of the cohorts studied had broadly neutralizing activity and only 3 out of 181 EC screened in the cohort were used in the study (Scheid et al., 2009). Thus, the majority of EC do not have broadly neutralizing antibodies and it is not clear that this activity plays a significant control in the control of viral replication. In fact, Deeks et al. (2006) showed a positive correlation between plasma viral load and titers of neutralizing antibody to heterologous virus suggesting that the development of these antibodies was a consequence of viral replication.

Perhaps the most important question in defining the role of humoral immunity in the elite conrol of HIV-1 infection is whether or not antibodies from EC are capable of neutralizing contemporaneous autologous virus. Mahalanabis et al. (2009) compared neutralizing activity in 2 ES and 3 patients with viral loads ranging from 695 copies/ml to 27,500 copies/ml over different time points. They found that at each time point, *env* clones from the viremic patients had varying sensitivity to autologous plasma. In contrast, while a similar pattern was seen with 1 EC, *env* clones from the other EC were uniformly resistant to neutralization by autologous plasma at all time points studied. Bailey et al. (2006b) compared neutralization of autologous Env in 9 EC, 7 viremic progressors and 9 patients in suppressive HAART regimens. They showed that although the degree of plasma and proviral *env* diversity in EC was significantly lower than in the other patients, there was no significant difference in the titers of Nab to autologous Env in all 3 patient groups. Furthermore they showed in another study that a very low titer of Nab to autologous virus was present at 12 months after infection in an EC (Bailey et al., 2007). This data suggests that Nabs do not a play a significant protective role in either the early or chronic phase of elite control of viral replication.

## 5. The role of CD4+ T cell mediated adaptive immunity

CD4+ T cells are the key regulators of the adaptive immune response. They provide help to B cells and CD8+ T cells and thus play an important role in the humoral and cytotoxic response to pathogens. They also play a key role in down modulating immune responses thereby preventing chronic inflammation. CD4+ T cells are the natural targets of HIV-1 infection and it is likely that the loss of these cells, especially HIV-specific CD4+ T cells (Douek et al., 2002) leads to the collapse of the immune response in patients with progressive disease. While it has been shown that there is no correlation between the magnitude of the HIV-specific CD4+ T cell response and protection from progressive HIV-1 disease (Betts et al., 2001), there are important qualitative differences between EC and viremic CP in CD4+ T cell responses.

An important study by Rosenberg et al. (1997) demonstrated that CD4+ T cells from LTNP were capable of proliferating upon stimulation with HIV antigens. The loss of this HIV-specific CD4+ T cell proliferation response has recently been shown to correlate with the loss of immune control in EC (Dyer et al., 2008), although prior studies suggest that the loss of this proliferative response can be an effect rather than a cause of viral replication in patients with progressive disease (McNeil et al., 2001; Blankson et al., 2002). EC have been shown to have a significantly higher percentage of CD4+ T cells that secreted both IL-2 and IFN-γ in response to HIV-1 antigens than patients with progressive disease (Emu et al., 2005, 2008; Pereyra et al., 2008). Recent work by Potter et al. (2007) suggest that

the degree of proliferation and IL-2 secretion in response to HIV antigens was greater in ES than in patients on suppressive HAART regimens. In contrast to these findings, Tilton and colleagues found no difference in the IL-2 secretion or proliferation response to HIV antigens in CD4+ T cells from EC versus patients on suppressive HAART regimens (Tilton et al., 2007). They also showed that the proliferative defect seen in viremic patients was due to diminished IL-2 secretion and could be reversed by the addition of this cytokine. Additionally, they showed that the response to HIV antigens in EC and patients on HAART was similar to the responses made against other viral antigens. Further experiments will be needed to resolve the different conclusions reached from these experiments.

While studies have focused mainly on cytokine secretion and proliferation by HIV-specific CD4+ T cells, it appears that these cells may also be capable of directly inhibiting viral replication. Unstimulated CD4+ T cells from an EC have been shown to kill autologous cells pulsed with Gag peptides as well as an infected cell line at high effector to target ratios (Norris et al., 2004). A similar phenomena has recently been seen with Gag and Nef-specific CD4 cell clones from monkeys that spontaneously control SIV replication (Sacha et al., 2009). Thus it appears that in some cases, EC CD4+ T cells can have a direct antiviral effect.

The role of clonal exhaustion in HIV-specific CD4+ T cells has been addressed in several studies. The regulatory molecule CTLA-4 was shown to be over-expressed in HIV-specific CD4+ T cells from patients with primary HIV-1 disease (Zaunders et al., 2006; Kaufmann et al., 2007), chronic progressors (Kaufmann et al., 2007), and patients on suppressive HAART regimens (Kaufmann et al., 2007), but not EC (Zaunders et al., 2006; Kaufmann et al., 2007). CTLA-4 blockade significantly improved the proliferative responses of HIV-specific CD4+ T cells in viremic CP (Kaufmann et al., 2007) which may partially explain the superior proliferative responses in EC. Interestingly, HIV-specific CD4+ T cells from EC with viral loads of <0.2 copies/ml had significantly lower levels of CTLA-4 than cells from EC with viral loads between 5 and 46 copies/ml suggesting that even very low levels of HIV-1 replication will lead to the upregulation of CTLA-4 (Kaufmann et al., 2007).

Regulatory CD4+ T cells (Tregs) are a subset of T cells that express the transcription factor FOXP3 and are involved in the modulation of the inflammatory response to many pathogens. While the related transcription factor FOXO3 has been shown to play a role in the persistence of CD4+ T cells in EC (van Grevenynghe et al., 2008), the role of FOXP3 expressing Tregs in HIV-1 infection remains unclear. Some studies have documented depletion of these cells and have suggested that this contributes to immune activation in chronically infected individuals whereas other studies have suggested that lower levels of these regulatory cells in non progressive disease could potentially explain the enhanced HIV-specific immunity seen in these individuals (Seddiki and Kelleher, 2008). One study found comparable levels of Tregs in EC and patients with primary HIV-1 disease (Zaunders et al., 2006). Chase and colleagues showed that EC had significantly higher levels of Tregs than viremic patients and patients on suppressive HAART regimens and documented a correlation between these levels and the level of activated CD4+ T cells (Chase et al., 2008). In spite of a higher frequency of Tregs, EC have higher levels of immune activation on CD8+ T cells than uninfected patients (Andrade et al., 2008; Hunt et al., 2008) and patients on suppressive HAART regimens (Hunt et al., 2008). EC also have superior HIV-specific CD8+ T cell responses as will be outlined below. Thus it appears that Tregs mediate a very fine balance between non-specific immune activation and HIV-specific immunity in EC.

## 6. The role of CD8+ T cell mediated adaptive immunity

CD8+ T cells are cytotoxic T cells (CTLs) that target and kill cells that are infected with intracellular pathogens such as viruses. Peptides from intracellular pathogens are processed into peptides that are eventually presented by Class I MHC molecules on the surface of the host cells. The peptides are recognized as being foreign, and thus the infected cells are killed by CTL in order to limit the extent of the infection. There have been multiple studies that have suggested a role for HIV-specific CD8+ T cells in elite control. Every cohort of EC has reported an over-representation of HLA-B*57 and/or HLA-B*27 alleles (Migueles et al., 2000; Lambotte et al., 2005; Emu et al., 2008; Pereyra et al., 2008; Han et al., 2008; Sajadi et al., 2009; Migueles et al., 2008). Furthermore SNPs associated with the HLA-B*57 allele and/or the HLA-C promoter have been consistently associated with low viral loads in multiple genome wide association studies (Fellay et al., 2007; Catano et al., 2008; Dalmasso et al., 2008; Limou et al., 2009; Shrestha et al., 2009; van Manen et al., 2009).

Because class I molecules present antigens to CD8+ T cells, it has seemed reasonable to assume that class I HLA findings was a reflection of the importance of HIV-specific CD8+ T cells. Evidence for this hypothesis includes a recent study where the MamuB*08 class I molecule, which is over-represented in macaques that that achieve EC status, was shown to bind peptides that were similar in structure to those bound by the protective human HLA-B*2705 allele (Loffredo et al., 2009). This finding suggests that the presentation of critical viral peptides to HIV-specific CD8+ T cells by protective HLA alleles leads to a distinct immune response in EC rather than the HLA-molecules being markers for some other protective factor.

Studies have suggested that CD8+ T cells specific for immunodominant peptides presented by HLA-B*27 (Almeida et al., 2007) and HLA-B*57 (Jansen et al., 2005) are activated at very low peptide concentrations (high functional avidity) which may contribute to the control of viral replication. It is not clear why some patients with these protective alleles become CP (Migueles et al., 2000), but it has been recently shown that replication-competent isolates cultured from EC are as effective as isolates from CP in downregulating surface HLA-A and B molecules (Nou et al., 2009), thus the qualitative differences in HIV-specific CD8+ T cells are probably not due to differences in the levels of class I molecules on the surface of infected CD4+ T cells.

While there is no correlation between the frequency of HIV-specific CD8+ T cells and the control of viral replication (Addo et al., 2003), multiple studies have suggested that qualitative differences in HIV-specific CD8+ T cell are associated with elite control. A significantly higher percentage of HIV-specific CD8+ T cells from ES make IL-2 than CD8+ T cells from patients with progressive disease (Emu et al., 2005, 2008; Pereyra et al., 2008). Betts and colleagues looked at 5 different effector functions and showed that HIV-specific cells from EC are more likely to secrete multiple cytokines and undergo degranulation than cells from patients with progressive disease (Betts et al., 2006). CD8+ T cells that have more than one of these functions have been described as “polyfunctional”. Interestingly, there was not a marked increase in the number of polyfunctional cells after the initiation of HAART in a subset of the progressors implying that the defect in these cells was not due to ongoing viral replication alone (Betts et al., 2006). Ferre et al. (2009) have shown that these polyfunctional cells are not just restricted to the peripheral blood, but are seen in mucosal tissue as well. EC were found to have significantly higher levels of polyfunctional HIV-specific CD8+ T cells in this important compartment which is thought to be a potential site of ongoing viral replication.

Studies using single copy assays have shown that EC and patients on suppressive HAART regimens have similar viral loads (Dinoso et al., 2008; Migueles et al., 2008; Hatano et al., 2009). In spite of similar levels of viremia, CD8+ T cells from these patients behave very differently. Migueles and colleagues compared T cell function in HLA-B*57+ EC, and patients on suppressive HAART regimens and showed that HIV-specific CD8+ T cell proliferation was seen only in EC (Migueles et al., 2002). Other studies have shown

that this proliferation is dependent on IL-2 secretion (Zimmerli et al., 2005) and is associated with high levels of telomerase activity (Lichterfeld et al., 2008). The lack of proliferative responses in HIV-specific CD8+ T cells from patients on HAART is in contrast to the proliferative responses of HIV-specific CD4+ T cell seen in these patients (Tilton et al., 2007). Thus one interpretation of the results is that the qualitatively superior responses seen in EC CD8+ T cells are the cause rather than an effect of the control of viral replication.

A study in SCID mice showed that the engraftment of CD8+ T cells from EC could restrict viral replication in vivo (de Quiros et al., 2000). It has also recently been shown that unstimulated CD8+ T cells from EC are capable of controlling HIV-1 replication in autologous CD4+ T cells whereas CD8+ T cells from viremic patients or patients on suppressive HAART regimens have a minimal effect on viral replication (Saez-Cirion et al., 2007; Saez-Cirion et al., 2009). This suppression is contact dependent and thus is not due to cytokine secretion alone (Saez-Cirion et al., 2007). Depletion of Gag-specific (but not Nef-specific) CD8+ T cells abrogates this suppressive activity (Saez-Cirion et al., 2009) which is consistent with other studies showing that CD8+ T cells from EC are more likely to target Gag than CD8+ T cells from patients with progressive disease (Emu et al., 2008; Pereyra et al., 2008; Saez-Cirion et al., 2009). A recent study has suggested that this impressive control of viral replication is probably due to enhanced lytic granule loading in CD8+ T cells from EC which leads to a marked increase in the delivery of granzyme B to target cells and thus more effective killing of HIV-1 infected cells (Migueles et al., 2008). Interestingly, the induction of proliferation in CD8+ T cells from patients with progressive disease led to acquisition of efficient lytic granule loading and killing of HIV-infected target cells via the granzyme B mediated pathway suggesting that the defect seen in cells from patients with progressive disease is reversible (Migueles et al., 2008) and that therapeutic vaccination may be a feasible goal.

While these studies suggest that HIV-specific CD8+ T cells play a key role in the control of viral replication, it is also clear that CD8+ T cells are unlikely to completely explain the elite control of HIV-1 infection. It has been shown that patients positive for the protective HLA-B*57 alleles control viral replication shortly after infection (Altfeld et al., 2003, 2006; Bailey et al., 2007). In one case a patient with replication-competent virus had a negative viral load within 3 months of a documented negative HIV test (Bailey et al., 2007). Furthermore, this patient had a frequency of latently infected cells of 0.05 infectious units per million at 6 months post-infection (Bailey et al., 2007), a level that is more than a log lower than the median value seen in patients on suppressive HAART regimens (Finzi et al., 1999; Siliciano et al., 2003) and 2 logs lower than the level typically seen in patients with primary HIV-1 disease (Blankson et al., 2000). The data suggests very rapid clearance and lower peaks of viremia during primary disease in EC. Virus specific CD8+ T cell responses are usually not seen until after peak viremia (Borrow et al., 1994; Koup et al., 1994), so these cells alone may not explain the rapid control of viremia.

It is also notable that some EC have very low frequencies of HIV-specific CD8+ T cell responses (Emu et al., 2008; Pereyra et al., 2008; Saez-Cirion et al., 2009) and cells from these patients generally have poor HIV-1 suppressive activity in vitro (Saez-Cirion et al., 2009). Finally, it has been shown that virus isolated from HLA-B*57 positive LTNP (Navis et al., 2007, 2008) and EC (Migueles et al., 2003; Bailey et al., 2006a, 2009; Miura et al., 2009b, 2009a) have significant levels of escape mutations. Studies that have performed direct comparisons of plasma and proviral genes (Bailey et al., 2006a, 2009) have shown that while escape mutations are rare in resting CD4+ T cells, virtually every plasma clone amplified from every ES has escape mutations in at least 2 HLA-B*57 restricted epitopes (Bailey et al., 2006b). These escape mutations occur during primary infection in HLA-B*57 (Goonetilleke et al., 2009) and HLA-B*5801 (O'Connell et al., in press) positive patients with low levels of viremia further calling into question how such rapid control is achieved in these individuals. It is clear that some of these escape mutations have a negative impact on viral fitness (Martinez-Picado et al., 2006; Brockman et al., 2007; Crawford et al., 2009; Boutwell et al., 2009) and reversion is seen when virus is transmitted to patients lacking these protective alleles (Leslie et al., 2004; Crawford et al., 2009), thus the diminished fitness of escape mutants can potentially contribute to the control of viremia. However, the same escape mutations are seen in patients with progressive disease and while compensatory mutations that restore fitness of these mutants were more commonly seen in HLA-B*57 EC with progressive disease compared to HLA-B*57 ES in one study (Brockman et al., 2007), another study found no significant difference in the frequency of these compensatory mutations in plasma viral clones amplified from EC and progressors (Miura et al., 2009b). Thus it appears that while HIV-specific CD8+ T cells play an important role in the maintenance of the control of viral replication in some EC, there may be other important factors that contribute to the acute and chronic phases of elite suppression.

How can we apply what we have learned from EC towards the design of a HIV-1 vaccine? The presence of replication-competent virus in the latent reservoir of EC suggests that eradication of the virus is not necessary to achieve control of HIV-1 infection. We also know that while high titers of broadly neutralizing antibody would be ideal for the prevention of HIV-1 infection, this is not necessary for the suppression of viral replication in patients who are already infected. It appears that virologic control is achieved shortly after primary infection in EC, and this might be crucial, as it may prevent the loss of HIV-specific CD4+ T cells and prevent clonal exhaustion of CD4+ and CD8+ T cells. While we do understand the innate immune response that may be responsible for this early control of viral replication in EC, multiple studies have shown that the early initiation of HAART in patients with progressive disease is associated with improved function in multiple parameters of the HIV-specific immune response (Rosenberg et al., 2000; Oxenius et al., 2000; Schito et al., 2001; Lecuroux et al., 2009a, 2009b). Thus one strategy may be to initiate HAART during primary infection and then to proceed with therapeutic vaccination in order to enhance the adaptive HIV-specific immune response.

The bulk of the evidence gathered to date favors a strong role of HIV-specific CD8+ T in the control of viral replication and a major goal of vaccination should be to develop CD8+ T cells that target the same critical immunodominant epitopes that are targeted by HLA-B*57 and HLA-B*27 positive EC. Furthermore, these HIV-specific CTL should be polyfunctional CD8+ T cells that are capable of cytokine secretion, proliferation and efficient granzyme B loading, leading to effective killing of infected CD4+ T cells and macrophages. While we are still far away from achieving the goal of an HIV-1 vaccine, the study of EC has given us a blueprint for what an effective therapeutic vaccine should look like.

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