Title
A low T regulatory cell response may contribute to both viral control and generalized immune activation in HIV controllers.

Permalink
https://escholarship.org/uc/item/7d10t0d5

Journal
PloS one, 6(1)

ISSN
1932-6203

Authors
Hunt, Peter W
Landay, Alan L
Sinclair, Elizabeth
et al.

Publication Date
2011-01-31

DOI
10.1371/journal.pone.0015924

Peer reviewed
A Low T Regulatory Cell Response May Contribute to Both Viral Control and Generalized Immune Activation in HIV Controllers

Peter W. Hunt1*, Alan L. Landay2, Elizabeth Sinclair1, Jeffrey A. Martinson2, Hiroyu Hatano1, Brinda Emu1, Philip J. Norris1,3, Michael P. Busch1,3, Jeffrey N. Martin1, Cicely Brooks2, Joseph M. McCune1, Steven G. Deeks1

1 Departments of Medicine and Laboratory Medicine, University of California San Francisco, San Francisco, California, United States of America, 2 Department of Immunology/Microbiology, Rush University Medical Center, Chicago, Illinois, United States of America, 3 Blood Systems Research Institute, San Francisco, California, United States of America

Abstract

HIV-infected individuals maintaining undetectable viremia in the absence of therapy (HIV controllers) often maintain high HIV-specific T cell responses, which has spurred the development of vaccines eliciting HIV-specific T cell responses. However, controllers also often have abnormally high T cell activation levels, potentially contributing to T cell dysfunction, CD4+ T cell depletion, and non-AIDS morbidity. We hypothesized that a weak T regulatory cell (Treg) response might contribute to the control of viral replication in HIV controllers, but might also contribute to generalized immune activation, contributing to CD4+ T cell loss. To address these hypotheses, we measured frequencies of activated (CD38+ HLA-DR+), regulatory (CD4+CD25+CD127dim), HIV-specific, and CMV-specific T cells among HIV controllers and 3 control populations: HIV-infected individuals with treatment-mediated viral suppression (ART-suppressed), untreated HIV-infected “non-controllers” with high levels of viremia, and HIV-uninfected individuals. Despite abnormally high T cell activation levels, controllers had lower Treg frequencies than HIV-uninfected controls (P = 0.014). Supporting the propensity for an unusually low Treg response to viral infection in HIV controllers, we observed unusually high CMV-specific CD4+ T cell frequencies and a strong correlation between HIV-specific CD4+ T cell responses and generalized CD8+ T cell activation levels in HIV controllers (P<0.001). These data support a model in which low frequencies of Tregs in HIV controllers may contribute to an adaptive effective immune response, but may also contribute to generalized immune activation, potentially contributing to CD4 depletion.

Citation: Hunt PW, Landay AL, Sinclair E, Martinson JA, Hatano H, et al. (2011) A Low T Regulatory Cell Response May Contribute to Both Viral Control and Generalized Immune Activation in HIV Controllers. PLoS ONE 6(1): e15924. doi:10.1371/journal.pone.0015924

Editor: Maria Ostrowski, University of Toronto, Canada

Received July 28, 2010; Accepted November 30, 2010; Published January 31, 2011

Copyright: © 2011 Hunt et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported in part by the UCSF/Gladstone Center for AIDS Research (P30 AI27763, P30 MH59037); NIAID (AI065244, AI055273, AI44595, AI067654, AI075981, and AI-76174); the Center for AIDS Prevention Studies (P30 MH62246); the UCSF Clinical and Translational Science Institute (UL1 RR024131-01); the CFAR Network of Integrated Clinical Sciences (SR24A0067039); the Ragon Institute of MGH, MIT, and Harvard; and American Foundation for AIDS Research (106710-40-RGRL). JMM is a recipient of a grant (DPI OD00329) from the NIH Director’s Pioneer Award Program, part of the NIH Roadmap for Medical Research. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: phunt@php.ucsf.edu

Introduction

The HIV vaccine field has returned “back to basics” after a T cell-mediated immunity vaccine recently failed to prevent HIV infection and actually increased the risk of infection in important subgroups of individuals [1]. Part of this process is a re-examination of the mechanisms by which some HIV-infected individuals spontaneously control viral replication in the absence of antiretroviral therapy. These HIV controllers represent fewer than 1% of chronically HIV-infected individuals and maintain clinically undetectable plasma HIV RNA levels (operationally defined as <75 copies/ml) in the absence of antiretroviral medications [2,3,4,5]. Several functional immunologic and host genetics studies suggest that high levels of HIV-specific CD4+ and CD8+ T cells with preserved function are likely to play an important role in the suppression of viral replication in most of these individuals [6,7,8,9,10,11,12,13,14,15,16,17,18,19], observations which have spurred the development of T cell immunity vaccines for HIV. However, the mechanisms of viral control in these individuals are likely to be heterogeneous as many HIV controllers lack a protective HLA type, have very low frequencies of HIV-specific T cells, or maintain control of viral replication even after documented escape from HLA-restricted epitopes [14,20,21,22].

It is important to recognize this heterogeneity as some mechanisms of viral control may prevent both initial infection and clinical progression better than others. For example, high T cell activation and low regulatory T cell (Treg) responses in highly exposed HIV-uninfected individuals have been consistently associated with an increased risk of subsequent HIV infection [23,24,25,26,27]. Higher T cell activation has also been independently associated with more rapid CD4+ T cell decline and clinical progression to AIDS in untreated HIV-infected individuals [28,29,30,31,32,33]. This potentially harmful effect of...
activation has even been observed among controllers [34]. Persistent immune activation in HIV controllers may also contribute to accelerated atherosclerosis and other non-AIDS morbidities linked to inflammation [35]. Understanding why some mechanisms of viral control are associated with negative inflammatory consequences is therefore an important issue for HIV vaccine development.

We hypothesized that an unusually low Treg response to viral infection might allow some HIV controllers to maintain strong antiviral immune responses at the cost of at the cost of abnormally high generalized immune activation, potentially contributing to CD4+ T cell decline even in the absence of clinically detectable viremia. To address these hypotheses, we measured frequencies of activated (CD38+ HLA-DR+), regulatory (CD4+CD25+CD127dim), HIV-specific, and CMV-specific T cells in a large cohort of HIV controllers. We compared these data to those observed in three well-characterized control populations: HIV-infected individuals with treatment-mediated viral suppression, untreated HIV-infected “non-controllers” with high levels of viremia, and HIV-uninfected individuals.

**Results**

**Characteristics of participants**

A total of 52 HIV controllers with plasma HIV RNA levels <75 copies/ml in the absence of antiretroviral therapy, 176 ART-suppressed participants, 72 untreated HIV-infected non-controllers with plasma HIV RNA levels >10,000 copies/ml, and 38 HIV-uninfected participants contributed to these studies. Most were men between 40 and 50 years of age, although compared to other HIV-infected groups, HIV controllers were more likely to be women (P = 0.006, Table 1). The HIV controllers were also much more likely to be hepatitis C virus (HCV) sero-positive than the other HIV-infected groups (70% vs. 38%, P<0.001). While all HIV controllers had plasma HIV RNA levels <75 copies/ml, 19 (37%) had an episode of a clinically measurable plasma HIV RNA level >75 copies/ml in the previous year. While the HIV controllers had significantly higher median CD4+ T cell counts than the ART-suppressed (683 vs. 449 cells/mm³, P<0.001) and the non-controllers (683 vs. 251 cells/mm³, P<0.001), 9 HIV controllers (17%) had CD4+ T cell counts below 350 cells/mm³ and 4 (7%) met the clinical definition of AIDS (one with Kaposi’s sarcoma and three with CD4+ T cell counts persistently <200 cells/mm³) despite maintaining viral suppression in the absence of therapy.

HIV controllers have low Treg frequencies despite higher T cell activation

We and others have previously reported that most HIV controllers maintain strikingly high frequencies of CD4+ and CD8+ T cells producing interferon (IFN)-γ and interleukin (IL)-2 in response to HIV Gag peptides [14,19,22], consistent with their potential role in the control of viral replication. However, as our group has recently reported in a smaller subset of participants (n = 30) [34], HIV controllers also had significantly higher frequencies of activated (CD38+ HLA-DR+) CD8+ T cells (Figure 1A) and CD4+ T cells (Figure 1B) than HIV-uninfected participants (P<0.001 for both), even when restricting to HCV-uninfected individuals (P<0.001). HIV controllers also had higher frequencies of activated CD8+ T cells than ART-suppressed participants (P = 0.017), even after adjustment for HCV sero-status, CD4+ T cell count, and gender (P = 0.056). As we have previously reported [34], higher frequencies of activated CD4+ and CD8+ T cells were associated with greater CD4+ T cell depletion in HIV controllers (P<0.001 for both, Figures S1A and S1B).

We hypothesized that a low Treg response to HIV infection might explain why most HIV controllers maintain high HIV-specific T cell responses but also high generalized T cell activation levels. To assess this possibility, we sampled cryopreserved peripheral blood mononuclear cells (PBMC) from 20 HIV controllers, 20 ART-suppressed, and 20 untreated non-controllers, and 34 healthy HIV–uninfected controls and compared the frequencies of CD25+CD127dim CD4+ Tregs between groups. Despite having higher frequencies of activated CD4+ and CD8+ T cells than HIV-uninfected controls, the HIV controllers had a lower median frequency of Tregs (3.9% vs. 4.9%, P = 0.014, Figure 1C). The HIV controllers also had a lower median frequency of Tregs than the ART-suppressed (3.9% vs. 5.0%, P = 0.008) and non-controllers (3.9% vs. 6.8%, P<0.001). While there was no evidence for a difference in Treg frequencies by gender within either group, among both HIV-uninfected and HIV-infected individuals, lower CD4+ T cell counts were associated with higher frequencies of regulatory T cells (rho: -0.60, P<0.001). To account for differences in absolute CD4+ T cell counts, we compared absolute regulatory T cell counts between groups. While absolute regulatory T cell counts were similar between HIV-infected groups, the HIV controllers continued to have a lower median CD25+CD127dim regulatory CD4+ T cell count than HIV-uninfected participants (33 vs. 40 cells/mm³, P = 0.004).

---

**Table 1. Characteristics of Participants Contributing to T Cell Activation and HIV-specific T Cell Response Analyses.**

| Characteristic                  | HIV-uninfected Controllers N = 38 Median (IQR) | HIV-infected Antiretroviral-treated N = 52 Median (IQR) | HIV-infected Untreated N = 72 Median (IQR) |
|--------------------------------|--------------------------------------------------|--------------------------------------------------------|------------------------------------------|
| Age, years                     | 43 (37 to 42)                                    | 48 (45 to 52)                                         | 46 (41 to 52)                             |
| Female gender, no. (%)         | 8 (22)                                           | 16 (31)                                               | 28 (16)                                  |
| CD4 count, cells/mm³           | -                                                | 683 (466 to 942)                                      | 449 (302 to 652)                         |
| Plasma HIV RNA level, log₁₀ copies/ml | -                                                   | <1.9                                                  | <1.9                                    |
| Hepatitis C seropositive, no. (%) | -                                                   | 24 (71)                                               | 47 (27)                                  |
| Duration of HIV Diagnosis, years | -                                                 | 16 (10 to 19)                                         | 13 (8 to 17)                             |

VL: Plasma HIV RNA Level.

1 Hepatitis C virus serology was unavailable for 18 of 52 controllers.

doi:10.1371/journal.pone.0015924.0001
It is surprising that HIV controllers have lower Treg frequencies and counts than HIV-uninfected individuals since higher levels of antigen stimulation and inflammation would be expected to cause greater expansion of Tregs [36]. Supporting this hypothesis, higher plasma HIV RNA levels were strongly associated with higher frequencies of Tregs among HIV-infected non-controllers (rho: 0.72, P < 0.001). Furthermore, among HIV controllers, higher frequencies of regulatory T cells were associated with higher frequencies of activated CD4+ T cells (rho: 0.49, P = 0.03) and activated CD8+ T cells (rho: 0.46, P = 0.04, Figure 1D). Based on these latter observations, we would have expected to observe higher Treg frequencies in HIV controllers than in HIV-infected individuals as a consequence of greater antigen stimulation and T cell activation. The observation that HIV controllers actually have lower Treg frequencies than HIV-uninfected individuals thus suggests that HIV controllers have an unusually weak Treg response to HIV infection, potentially contributing to the high HIV-specific T cell responses and generalized T cell activation observed.

Strong relationship between adaptive HIV-specific immune response and generalized T cell activation in HIV controllers

Since unusually low Treg responses in HIV controllers might allow for both stronger adaptive HIV-specific immune responses and generalized T cell activation, we hypothesized that there would be a strong relationship between these two latter factors. Among HIV controllers, higher frequencies of CD4+ T cells producing both IFN-γ and IL-2 in response to stimulation with HIV Gag peptides were strongly associated with higher frequencies of activated CD4+ T cells (rho: 0.36, P = 0.012) and activated CD8+ T cells (rho: 0.55, P < 0.001, Figure 2A). Higher frequencies of HIV Env-specific CD4+ T cell responses were also associated with higher frequencies of activated CD8+ T cells (n = 28, P = 0.46, P = 0.014, Figure 2B). However, there was no evidence for a relationship between Pol-specific or Nef-specific CD4+ T cell responses and the frequency of activated CD4+ or CD8+ T cells. HIV controllers with higher plasma HIV-specific antibody levels (as assessed by de-tuned ELISA) also had higher frequencies of
activated CD4+ T cells (rho: 0.46, P = 0.025) and CD8+ T cells (rho: 0.60, P = 0.002, Figure 2D).

The frequency of HIV-specific CD8+ T cells was less consistently associated with the frequency of activated T cells. In general, there was little evidence for an association between the frequency of HIV-specific CD8+ T cells producing both IFN-γ and IL-2 and the frequency of activated CD4+ or CD8+ T cells. However, higher frequencies of activated CD8+ T cells tended to be associated with higher frequencies of CD8+ T cells producing IFN-γ but not IL-2 in response to HIV Nef (rho: 0.42, P = 0.025, Figure 2C), Pol (rho: 0.39, P = 0.045), and Gag peptides (rho: 0.22, P = 0.14).

HIV controllers also have high CMV-specific CD4+ T cell responses

We next hypothesized that an unusually low Treg response in HIV controllers might also contribute to higher adaptive immune responses directed at other chronic viral infections. We chose to focus on cytomegalovirus (CMV) since CMV is nearly ubiquitous in HIV infected individuals, is typically controlled to nearly undetectable levels in individuals with intact immune systems, yet elicits high frequencies of CMV-specific T cells even in HIV-uninfected individuals [37,38]. To address this, we compared CMV-specific T cell responses between HIV-uninfected but CMV-sero-positive controls, HIV controllers, and untreated HIV-infected participants with varying plasma HIV RNA levels (75–2,000, 2,001–10,000, and >10,000 copies/ml). The HIV controllers had higher CMV pp65-specific IFN-γ/IL-2+ T cell responses than HIV-uninfected controls (P < 0.001, Figure 3A). While HIV controllers had similar frequencies of pp65-specific IFN-γ/IL-2+ T cells as untreated HIV-infected participants maintaining low but detectable plasma HIV RNA levels between 75 and 2,000 copies/ml, they had significantly higher frequencies

Figure 2. Relationship between Adaptive HIV-specific Immune Responses and CD8+ T Cell Activation in HIV Controllers. The association between the frequency of activated (CD38-HLA-DR+) CD8+ T cells and the frequency of CD4+ T cells producing both IFN-γ and IL-2 after stimulation with overlapping HIV Gag (A) or HIV Env peptides (B), CD8+ T cells producing only IFN-γ after stimulation with overlapping Nef peptides (C), and plasma HIV-specific antibody levels (as assessed by de-tuned ELISA, D) were assessed among HIV Controllers. The curves in each plot represent best-fit linear or quadratic regression models using untransformed data.
doi:10.1371/journal.pone.0015924.g002

activated CD4+ T cells (rho: 0.46, P = 0.025) and CD8+ T cells (rho: 0.60, P = 0.002, Figure 2D).
of pp65-specific IFN-γ T cells than HIV-infected participants with plasma HIV RNA levels >10,000 copies/ml (P = 0.003). Across all 4 groups of untreated HIV-infected participants, lower plasma HIV RNA levels were associated with higher pp65-specific CD4+ T cell frequencies (P = 0.001). Even after adjustment for age, HIV controllers continued to have higher pp65-specific CD4+ T cell responses than HIV-uninfected participants (P = 0.003) and untreated HIV-infected participants with plasma HIV RNA levels >10,000 copies/ml (P = 0.016). Notably, HIV controllers with the highest frequencies of pp65-specific CD4+ T cells also had the highest frequencies of Gag-specific CD4+ T cells (rho: 0.32, P = 0.024, Figure 3B). Similar trends were observed when comparing the frequency of CMV-specific IFN-γ+ IL-2+ CD4+ T cells across groups in a smaller subset of individuals (data not shown). There was no evidence for a consistent relationship between pp65-specific CD8+ T cell responses and plasma HIV RNA levels among untreated HIV-infected individuals.

Discussion

A wealth of data now suggest that most HIV controllers maintain control of viral replication at least in part through potent HIV-specific T cell responses [6,7,8,9,10,11,12,13,14,20,22], observations that have spurred the development of vaccines that elicit T cell responses against HIV. However, the mechanisms responsible for a strong HIV-specific T cell response in HIV controllers may not be without important consequences for the immune system. As our group recently reported, most HIV controllers have abnormally high levels of immune activation, which is associated with significant CD4+ T cell depletion and even AIDS despite continued control of viral replication [34]. In the current study, we have expanded upon this prior work and assessed potential mechanisms to explain this paradox. First, despite abnormally high T cell activation levels, HIV controllers have significantly lower Treg frequencies than HIV-uninfected individuals. Second, we observed a strikingly strong relationship between adaptive HIV-specific CD4+ T cell and antibody responses and generalized T cell activation in HIV controllers. Third, we observed unusually high CMV-specific CD4+ T cell responses in HIV controllers, suggesting that their ability to mount strong T cell responses to chronic viral infections may not be specific for HIV. Collectively, these observations suggest that a low Treg response may allow many HIV controllers to maintain viral control with a strong cytotoxic HIV-specific T cell response, but might also contribute to the negative inflammatory consequences of generalized T cell activation in this setting (Figure 4).

Multiple mechanisms have been proposed to explain why HIV controllers maintain low to undetectable levels of viral replication in the absence of therapy. While it is possible that some HIV controllers may simply be infected with defective viruses [39], most harbor replication competent viruses that lack gross deletions or lethal mutations [40,41]. Several lines of evidence suggest an important role of HIV-specific T cells in the control of viral replication. For example, most HIV controllers maintain unusually high frequencies of HIV-specific CD4+ and CD8+ T cells [6,7,8,9,10,11,12,13,14,19], as well as HIV-specific CD8+ T cells with greater proliferative and cytotoxic potential [8,12,42]. While strong HIV-specific T cell responses could conceivably be a consequence of poor viral fitness [43,44], HIV controllers are highly enriched for protective class I HLA alleles (i.e., B5701) and polymorphisms associated with HLA C expression [15,16,17,18], suggesting that CD8+ T cell responses may play an important role in the control of HIV replication. Some HIV controllers also have high frequencies of CD4+ T cells with cytotoxic activity [43,46]. However, many HIV controllers lack a protective HLA type, have very low frequencies of HIV-specific T cells, or maintain control of viral replication even after documented escape from HLA-restricted
Figure 4. Theoretical Model to Describe Positive and Negative Consequences of Low Treg Frequencies in HIV Controllers. A theoretical model to describe the potential positive and negative consequences of low Treg frequencies in HIV controllers is presented. While a low Treg response might increase HIV-specific T cell responses, contributing to the clearance of HIV-infected cells and the maintenance of extremely low levels of viral replication, a low Treg response might also increase generalized T cell activation, contributing to CD4+ T cell decline and other inflammation-associated comorbidities even in the presence of very low levels of viral replication.

doi:10.1371/journal.pone.0015924.g004

It is important to acknowledge this heterogeneity in the mechanisms of viral control in HIV controllers as some mechanisms are likely to be associated with more negative inflammatory consequences than others. While other cohorts have not observed increased T cell activation levels in HIV controllers [50,51], these studies either included individuals with nef-deleted viruses or only included HIV controllers maintaining normal CD4+ T cell counts. When selecting HIV controllers solely on the basis of their ability to control viral replication, it is clear that some controllers eventually progress to significant levels of CD4+ T cell depletion [5,52,53], and these individuals have the highest T cell activation levels [34]. In a recent study, we also observed that HIV controllers with the highest HIV-specific CD4+ T cell frequencies and antibody levels had the highest levels of generalized T cell activation and the greatest degree of CD4+ T cell depletion. Thus, the HIV-specific immune response and generalized T cell activation are tightly linked in HIV controllers and these relationships appear to be stronger than those observed in untreated HIV-infected individuals with high levels of viral replication [54,55]. While we cannot exclude the possibility that higher adaptive immune responses are simply a consequence of greater degrees of low-level viral replication - particularly in lymphoid tissues, differences between HIV controllers in the degree of adaptive immune responses and T cell activation may well reflect host differences in the immune response elicited by any given level of virus replication. The extent of microbial translocation may be one factor modulating the response to low-level HIV replication. As we reported previously, most HIV controllers have abnormally high plasma lipopolysaccharide levels [34], which might drive generalized immune activation, but also serve as an adjuvant for HIV-specific T cell responses, particularly in gut-associated lymphoid tissue where the majority of HIV replication is thought to occur.

Alternatively, HIV controllers may be enriched for host genetic factors associated with strong innate and/or weak Treg responses to viral infection. Indeed, we found that HIV controllers had significantly lower frequencies of CD25+CD127dim CD4+ Tregs in peripheral blood than HIV-uninfected individuals despite much higher levels of T cell activation. While we cannot exclude the possibility that HIV controllers preferentially retain Tregs in lymphoid tissues, a recent study also found low frequencies of Tregs in tissues of HIV controllers [56]. While the specific mechanisms mediating the unusually low Treg frequencies in HIV controllers remain unclear, a low Treg response is likely to have competing effects in this setting. For example, several studies have argued that these cells are detrimental in HIV infection by inhibiting HIV-specific T cell responses [56,57,58,59,60,61], while others have argued that these cells are beneficial by reducing generalized T cell activation [62,63,64,65]. Inferring causal relationships is particularly challenging in cross-sectional studies of in vivo Treg frequency in HIV-infected individuals since Tregs may be induced and expanded by viral replication and resultant inflammation [36], but once induced, act to decrease inflammation. Accordingly, we observed that HIV controllers with higher levels of immune activation had higher frequencies of Tregs, suggesting that inflammation was driving the induction of Tregs. However, HIV controllers had lower Treg frequencies than HIV-uninfected individuals despite having much higher T cell activation, suggesting a strikingly low Treg response for the degree of immune activation observed. This unusually low Treg response in HIV controllers is therefore likely to be a significant contributor to the high generalized T cell activation and HIV-specific T cell responses observed. These results are consistent with a recent report of decreased inhibitory immunoregulatory receptor CTLA-4 expression on CD4+ T cells in HIV controllers [66].

Our results differ from another recent report describing preserved Treg frequencies (as defined by FoxP3 expression) in the peripheral blood of a much smaller cohort of 12 HIV controllers [50]. However, FoxP3 can be expressed early in the activation of effector CD4+ T cells without any regulatory function [67,68,69,70,71,72], so the preserved FoxP3 expression described in that study may simply reflect the presence of recently
activated effector CD4+ T cells, particularly since the co-expression of CD25 and FoxP3 in CD4+ T cells was not presented. Low expression of CD127, as measured in our study, may help distinguish Tregs from activated T cells and is now routinely used with CD25 to quantify the frequency of Tregs with suppressor function [73,74,75]. It should be noted that among HIV-infected individuals with high levels of viral replication, gating on CD4+/CD25+/CD127dim may include some cells that do not express FoxP3 and thereby lack regulatory function [76]. However, Treg frequencies defined by CD4+/CD25+/CD127dim and CD4+/CD25+FoxP3+ are highly correlated in HIV-infected individuals with undetectable plasma HIV RNA levels ($r = 0.91$, $P<0.001$) [76]. Thus, the low frequency of CD4+/CD25+/CD127dim cells we observed in HIV controllers relative to HIV-uninfected controls and ART-suppressed individuals (all groups with undetectable viremia) almost certainly reflects a low frequency of Tregs in HIV controllers. Lastly, even if HIV controllers had similar levels of Tregs to HIV-uninfected individuals as has been suggested in another recent report using HIV controller samples from the same cohort [77], they would still have unusually low Treg frequencies relative to the expansion of activated T cells observed.

Consistent with the hypothesis that HIV controllers are predisposed to a weak Treg response to chronic viral infections, we observed significantly higher CMV-specific CD4+ T cell responses in HIV controllers than non-controllers and HIV-uninfected individuals. While we cannot exclude the possibility that greater CMV shedding explains the higher CMV-specific CD4+ T cell responses in HIV controllers, CMV shedding tends to be lower in individuals with higher CD4+ T cell counts and lower plasma HIV RNA levels [78]. Thus, the expansion of CMV-specific CD4+ T cells in HIV controllers is unlikely to be driven by higher levels of antigen and is more likely to reflect a more robust proliferation of CD4+ T cells in response to CMV infection. HIV controllers co-infected with hepatitis C virus (HCV) might also exhibit stronger HCV-specific responses than individuals with higher levels of HIV replication [79]. While lower levels of HIV replication may allow for preservation of antigen-specific immune responses, the high CMV-specific CD4+ T cell frequency in HIV controllers relative to HIV-uninfected CMV-seropositive individuals cannot be explained by this mechanism alone. While another recent report suggested that HLA B5701+ elite controllers maintain similar CMV- and HCV-specific CD8+ T cell responses as non-controllers, CD4+ T cell responses were not assessed in that study [80], and epidemiologic data suggest that HIV controllers are much more likely to spontaneously clear HCV than viremic HIV-infected individuals and HIV-uninfected individuals infected with HCV [81].

In summary, we have observed that while most elite controllers maintain high HIV-specific T cell responses, most also have abnormally high generalized T cell activation levels, which may occasionally contribute to significant CD4 depletion even in the absence of clinically detectable viremia. Furthermore, those with the highest HIV-specific T cell responses have the highest levels of generalized immune activation, suggesting possible inflammatory consequences of T cell-mediated control of HIV replication. An unusually low regulatory T cell response to HIV infection may well explain this phenomenon. Perhaps the best immune response to HIV infection is one that maintains control of viral replication while minimizing negative inflammatory consequences. Some elite controllers are able to maintain this balance and understanding the mechanisms of control in these individuals is likely to have important implications for HIV vaccine research.

Materials and Methods

Participants

For comparison of HIV-specific immune responses and T cell activation levels. HIV-infected adults were sampled from the Study of the Consequences of the Protease Inhibitor Era (SCOPE), a clinic-based cohort of over 1000 chronically HIV-infected individuals at the University of California San Francisco. From this cohort, we evaluated three distinct groups of HIV-infected individuals: (1) HIV controllers, defined as HIV-seropositive individuals maintaining plasma HIV RNA levels <75 copies/ml in the absence of therapy (episodes of clinically detectable viremia in the previous year were allowed if they were followed by undetectable values); (2) “ART-suppressed” individuals maintaining plasma HIV RNA levels <75 copies/ml on antiretroviral therapy; and (3) untreated HIV “non-controllers” with plasma HIV RNA levels above 10,000 copies/mL. T cell activation data have been previously reported on 30 of the 52 HIV controllers and all of the ART-suppressed and untreated patients in the current report [34], HIV-specific T cell response data have also been reported on these individuals recently [14]. HIV-uninfected individuals were also sampled from a study of the immunologic determinants of atherosclerosis and have been reported on previously [14,82].

For comparisons of CMV-specific T cell responses between groups. In addition to the above participants, untreated HIV-infected participants with plasma HIV RNA levels between 75 and 10,000 copies/ml were sampled from the SCOPE cohort. HIV-negative individuals were also sampled from a trial of post-exposure prophylaxis following a non-occupational exposure to HIV [83]. Only CMV-seropositive HIV-negative participants were included in the analyses of CMV-specific T cell responses.

For comparison of Tregs between groups. Given limited PBMC availability, cryopreserved PBMC from different SCOPE participant-timepoints were sampled for the measurement of both Treg frequency and T cell activation levels in 20 HIV controllers, 20 HAART-suppressed participants, and 20 non-controllers. Only specimens on participants with CD4+ T cell counts >350 cells/mm$^3$ were selected for these analyses to ensure adequate overlap between groups. For the Treg analyses, cryopreserved PBMC were also sampled from 34 healthy HIV-uninfected controls from the AIDs Clinical Trials Group 5015 study [84].

Ethics Statement

All participants provided written informed consent and this research was approved by the institutional review board of the University of California, San Francisco.

Laboratory Studies

T cell activation. Freshly collected, EDTA-anticoagulated whole blood was analyzed by four-color flow cytometry on a Beckman Coulter Epics XL flow cytometer. Blood was stained on a Beckman Coulter Prep Plus and lysed on a Beckman Coulter TQ Prep. Activated (CD38+/HLA-DR+) T cells were identified with FITC-conjugated anti-HLA-DR, PE-conjugated anti-CD38 (both from BD Bioscience), PC5-conjugated anti-CD3 and PE-texas red conjugated anti-CD4 or CD8 (Beckman Coulter). The activation markers CD38 and HLA-DR were gated from the CD3+CD4+ or CD3+CD8+ cells on a 2-dimensional dot plot where quadrant gates, set on an isotype control, were used to define positive and negative populations. T cell activation levels were reported as the percentage of CD4+ and CD8+ T cells expressing both HLA-DR and CD38.
Cytokine flow cytometry. Fresh whole blood was stimulated with overlapping peptide pools (15-amino-acid peptides overlapping by 11 amino acids) of the HIV-1 p55 Gag, Pol, Nef, Env, or CMV pp65 protein (BD Biosciences, San Jose, CA) for 6 h in the presence of brefeldin A, as reported recently [14]. Unstimulated cells and superantigen staphylococcal enterotoxin B (Sigma Aldrich)-stimulated cells were used as negative and positive controls, respectively. Cells were fixed, permeabilized, and stained with FITC-conjugated anti-interferon (IFN)-γ, PE-conjugated anti-IL-2, APC-conjugated anti-CD3 (all BD Bioscience) and PC5-conjugated anti-CD4 (Beckman Coulter) and data was collected on a Becton Dickinson FACSCalibur. The fractions of CD4+ and CD8+ T cells secreting IFN-γ and/or IL-2 were determined using FlowJo software (TreeStar). In our primary analysis of CMV-specific T cell responses, we focused on cells that stained brightly for IFN-γ. The “IFN-γ bright” gate was set 3 decades above the IFN-γ-negative population in non-stimulated control, as previously described (representative flow plot depicted in Figure 1 from reference [85]). Cells were initially defined as lymphocytes based on forward- and side-scatter profiles. CD4+ and CD8+ anchor gates were drawn on the CD3+CD4+ and CD3+CD8+ populations, respectively. At least 10,000 CD3+CD4+ and CD3+CD8+ events were collected for the majority of subjects; data were excluded if <4,000 events were collected. Cytokine secretion levels in the negative control were subtracted to correct for nonspecific cytokine secretion.

Treg frequencies. Cryopreserved PBMC were evaluated using 4-color flow cytometry. Mouse anti-human monoclonal antibodies (CD4, CD8, CD25, CD45RO, and CD127) conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), PerCP, and allophycocyanin (APC) from BD Biosciences (San Jose, CA) or Coulter Immunology (Miami, FL) were used to stain the PBMC preparations. Non-specific antibody binding to Fc receptors was blocked by pre-incubation of the cells with Fcγ-receptor block (Miltenyi Biotec, Auburn, CA). All samples were evaluated within 24-hours of staining using a FACSCalibur™ flow cytometer. Logical gating was used to identify the frequency of T regulatory (CD4+CD25+CD127dim) T lymphocyte populations (Figure 1B) [73, 74, 75]. Results are expressed as the percentage of the parent CD4+ T cell population.

HIV Antibody Levels. A “de-tuned” enzyme immunoassay (Organon Tecnica Vironostika [OTV], BioMerieux) was used to measure semiquantitative HIV antibody levels on a subset of HIV controllers [86]. The OTV is a second-generation ELISA that detects both IgG and IgM antibodies to HIV-1 and is FDA-approved for diagnostic testing. The less sensitive modification involves testing 1:20,000 dilutions of plasma under abbreviated incubation conditions and calculating a standardized optical density (SOD) for each sample [87].

Supporting Information

Figure S1 (TIFF)

Acknowledgments

The authors would like to thank Dr. Dennis Hartigan-O’Connor for his thoughtful discussion of this work.

Author Contributions

Formulated the hypotheses, contributed to the design of the research, analyzed the data, interpreted the results, and wrote the manuscript: PWH. Contributed to framing the hypotheses, the design of the research, supervised the Treg measurements, and provided access to ACTG control samples: ALL. Performed the T cell activation and cytokine flow cytometry measurements: ES. Performed the Treg measurements: JAM CB. Provided access to the low-level viremia and HIV-specific antibody measurements, analyzed these data, and contributed to the interpretation of these measures: HH. Provided access to the cytokine flow cytometry data and contributed to the interpretation of these measures: BE. Supervised the HIV-specific antibody measurements and contributed to the interpretation of these results: PJJ MB. Provided access to patient samples and contributed to the design and analysis of the data: JNM. Contributed to framing the hypotheses and interpreting the results: JAM. Contributed to access to the low-level viremia and HIV-specific antibody measurements, contributed to the interpretation of these measures: ES. Performed the T cell activation and cytokine flow cytometry measurements: JAM CB. Provided access to patient samples and contributed to framing the hypotheses, designing the research, and interpreting the results: SGD.

References

1. Buchbinder SP, Mehrotra DV, Duer A, Fitzgerald DW, Mogg R, et al. (2008) Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomized, placebo-controlled, test-of-concept trial. Lancet 372: 1081–1089.
2. Hubert JB, Buragd M, Dassa E, Tamalet C, Devaux C, et al. (2000) Natural history of serum HIV-1 RNA levels in 330 patients with a known date of infection. The SEROCO Study Group. AIDS 14: 123–131.
3. Goudsmit J, Boggs JA, Jurriaans S, Schuitemaker H, Lange JM, et al. (2002) Naturally HIV-1 seroconverters with lowest viral load have best prognosis, but in time lose control of viriaemia. AIDS 16: 791–793.
4. Lambotte O, Boulaffa F, Madyer Y, Nguyen A, Goujard C, et al. (2005) HIV controllers: a homogeneous group of HIV-1-infected patients with spontaneous control of viral replication. Clin Infect Dis 41: 1053–1056.
5. Madyer Y, Boufassa F, Porter K, Meyer I, et al. (2005) Spontaneous control of viral load and CD4+ cell count progression among HIV-1 seroconverters. AIDS 19: 931–937.
6. Bettis MR, Nason MC, West SM, De Rosa SC, Migueles SA, et al. (2006) HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107: 4781–4789.
7. Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, et al. (2008) Lytic function, leading of CD8+ T cells, is required for HIV-infected cell elimination associated with immune control. Immunity 29: 1009–1021.
8. Potter SJ, Lacabaratz C, Lambotte O, Perez-Patrigeon S, Vinuet B, et al. (2007) Preserved central memory and activated effector memory CD4+ T-cell subsets in human immunodeficiency virus controllers: an ANRS EP66 study. J Virol 81: 13904–13915.
9. Bailey JR, Brennan TP, O’Connell KA, Siliciano RF, Blankson JN (2009) Evidence of CD8+ T-cell-mediated selective pressure on human immunodeficiency virus type 1 nef in HLA-B*57+ elite suppressors. J Virol 83: 88–97.
10. Emu B, Sinclair E, Favre D, Moreto WD, Huse P, et al. (2005) Phenotypic, Functional, and Kinetic Parameters Associated with Apparent T-Cell Control of Human Immunodeficiency Virus Replication in Individuals with and without Antiretroviral Treatment. J Virol 79: 14169–14178.
11. Harari A, Petitpierre S, Vallian F, Pantalos G (2004) Skewer representation of functionally distinct populations of virus-specific CD4+ T cells in HIV-1-infected subjects with progressive disease: changes after antiretroviral therapy. Blood 103: 966–972.
12. Saez-Girzon A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, et al. (2007) HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proc Natl Acad Sci U S A 104: 6776–6781.
13. Pereyra F, Addo MM, Kaufmann DE, Liu Y, Miura T, et al. (2008) Genetic and Functional, and Kinetic Parameters Associated with Apparent T-Cell Control of Human Immunodeficiency Virus Infection, but such responses are not always necessary for long-term virus control. J Virol 82: 5398–5407.

PLoS ONE | www.plosone.org 8 January 2011 | Volume 6 | Issue 1 | e15924
15. van Manen D, Kooststra NA, Boerse-Nunnink B, Handulle MA, van’t Wou AB, et al. (2009) Association of HLA-C and HCP5 genes with the clinical course of HIV-1 infection. AIDS 23: 19–28.
16. Fellay J, Shaanna KV, Ge D, Colombi S, Ledegerber B, et al. (2007) A whole-genome association study of major determinants for host control of HIV-1. Science 317: 944–947.
17. Catano G, Kulkarni H, He W, Marconi VC, Agan BK, et al. (2008) HIV-1 disease-influencing effects associated with ZRND1, HCP5 and HLA-C alleles are attributable mainly to either HLA-A10 or HLA-B57 alleles. PLoS ONE 3: e3636.
18. Limou S, Le Clerc S, Coulonges C, Carpentier W, Dina C, et al. (2009) Human immunodeficiency virus type 1 controllers but not noncontrollers maintain CD4 T cells coexpressing three cytokines. J Virol 81: 12071–12076.
19. van Manen D, Kootstra NA, Boeser-Nunnink B, Handulle MA, van’t Wout AB, et al. (2006) The role of immune activation in the pathogenesis of HIV-1 infection in a subset of elite suppressors. AIDS 22: 541–544.
20. Bailey JR, Williams TM, Siliciano RF, Blankson JN (2006) Maintenance of viral suppression in advanced human immunodeficiency virus type 1 infection is associated with low levels of CD4+ T cell activation. J Exp Med 203: 1357–1369.
21. Pereyra F, Addo MM, Kauffman DE, Liu Y, Miura T, et al. (2008) Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. J Infect Dis 197: 563–573.
22. Koenning S, Capogna SS, Begou B, Chennareddi L, Ge D, et al. (2007) Low-level CD4+ T cell activation is associated with low susceptibility to HIV-1 infection. J Immunol 178: 6117–6122.
23. Bousquet E, Chartrand L, Marchal V, Iero J, Leal J, et al. (2006) Reduced CD4 T cell activation and in vitro susceptibility to HIV-1 infection in uninfected subgroup of African. Retrovirology 3: 5.
24. Jerns W, Everse D, Borgen MV, Vuylsteke B, Maurice C, et al. (2006) Suppressed cell-mediated immune responses in HIV-exposed seronegative female sex workers. Clin Exp Immunol 143: 435–444.
25. Salkowski RJ, Purvis SF, Meyerson H, Osorio BM, O’Brien TR, et al. (2001) Characterization of high-risk HIV-1 seronegative hemophiliacs. Clin Immunol 99: 200–211.
26. Card CM, McLean WM, Wachhui C, Kimani J, Plummer FA, et al. (2009) Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)/CD25(+)/FOXp3(+) regulatory T cells. J Infect Dis 199: 1319–1323.
27. Giorgi JV, Ikeda RH, Matud JL, Yamashita TE, Mellors JW, et al. (2002) Relationship between the frequency of CD38(+)/CD25(-) regulatory T cells and the level of soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J Acquir Immun Defic Syndr 29: 361–365.
28. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, et al. (1999) Shorter survival in advanced human immunodeficiency virus type 1 infection is associated with disease progression. AIDS Res Hum Retroviruses 25: 183–191.
29. Giorgi JV, Hultin LE, Hultin PM, Detels R (2009) Regulatory T cells and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. J Virol 81: 2508–2518.
30. Bailey JR, O’Connell K, Yang HC, Han Y, Xu J, et al. (2008) Transmission of human immunodeficiency virus type 1 from a patient who developed AIDS to an elite suppressor. J Virol 82: 7395–7410.
31. Limou S, Le Clerc S, Coulonges C, Carpentier W, Dina C, et al. (2009) Human Immunodeficiency Virus (HIV)-Specific Cytotoxic T-Lymphocyte Activity in Sydney Blood Bank Cohort Patients Infected with nef-Defective HIV Type 1. Journal of Virology 83: 436–443.
32. Miura T, Broekman MA, Brumme ZL, Brumme CJ, Pereyra F, et al. (2009) HIV-associated alterations in replication capacity of chimeric NLR+3 viruses carrying gag-protein from elite controllers of human immunodeficiency virus type 1. J Virol 83: 146–149.
33. Kaufmann DE, Bailey PM, Sidney J, Wagner B, Norris PJ, et al. (2004) Comprehensive analysis of human immunodeficiency virus type 1-specific CD4+ T cell responses reveals marked immunodominance of gag and nef and the presence of broadly recognized peptides. J Virol 78: 4536–4547.
34. Saunders J, Dyer WB, Wang B, Munner ML, Miranda-Sakamura M, et al. (2004) Identification of circulating and mucosal specific CD4+ T lymphocytes with a cCR5+, cytoytic phenotype in an HIV-1 long-term nonprogressor and in CMV infection. Blood 103: 2230–2247.
35. Martin MP, Gao X, Lee JH, Wilson GW, Detels R, et al. (2002) Episitic interaction between KIR3DL1 and HLA-B delays the progression to AIDS. Nat Genet 31: 429–434.
36. Martin MP, Qi Y, Gao X, Yamada E, Martin JN, et al. (2007) Inmate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 39: 737–740.
37. Louril Sky, Deutsch S, Cicufri A, Roby D, Taiffe P, et al. (2008) In vitro whole-genome analysis identifies a susceptibility locus for HIV-1. PLoS Biol 6: e32.
38. Chase AJ, Yang HC, Zhang H, Blankson JN, Siliciano RF (2000) Preservation of FoxP3 regulatory T cells in the peripheral blood of human immunodeficiency virus type 1-infected elite suppressors correlates with low CD4+ T-cell activation. J Virol 82: 8307–8313.
39. Zauders J, Cunningham PH, Kelledge AR, Kauffmann GR, Jaramillo AB, et al. (1999) Potent antiretroviral therapy of primary human immunodeficiency virus type 1 (HIV-1) infection: partial normalization of T lymphocyte subsets and limited reduction of HIV-1 DNA despite clearance of plasma viremia. J Infect Dis 180: 320–329.
40. Grabar S, Selinger-Leneman H, Abgrall S, Plaixoux G, Weiss L, et al. (2009) Prevalence and comparative characteristics of long-term nonprogressors and HIV controller patients in the French Hospital Database on HIV. AIDS 23: 1163–1169.
41. Pereyra F, Palmer S, Miura T, Block BL, Wiegand A, et al. (2009) Persistent low-level viremia in HIV-1 elite controllers and relationship to immunologic markers. J Infect Dis 200: 189–196.
42. Chu TN, Justment JS, Sanford C, Hallahan CW, Planta MA, et al. (2004) Relationship between the frequency of HIV-specific CD8+ T cells and the level of CD38+CD4+CD8+ T cells in untreated HIV-infected individuals. Proc Natl Acad Sci U S A 101: 2464–2469.
43. Ho NH, Hultin LE, Mituyasu RT, Matud JL, Hausner MA, et al. (1993) Accumulation of CD4+ and CD8+ T lymphocytes with a cytotoxic phenotype in an HIV-1 long-term nonprogressor. J Immunol 169: 3400–3406.
44. Kurtner MS, Israelski D, Wolk SM, Yarasheski EK, Yarasheski CE, et al. (2003) Cytomegalovirus-specific T cells persist at very high levels during long-term nonprogression in HIV-infected patients. J Acquir Immun Defic Syndr 33: 356–362.
45. Kaufmann DE, Bailey PM, Siddiqi H, Storm HH, Scharf SM, et al. (2003) Serum total IgM and rheumatoid factors are attributable mainly to either HLA-A10 or HLA-B*57 alleles. PLoS ONE 3: e3636.
46. Martin MP, Qi Y, Gao X, Yamada E, Martin JN, et al. (2007) Inmate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 39: 737–740.
47. Louril Sky, Deutsch S, Cicufri A, Roby D, Taiffe P, et al. (2008) In vitro whole-genome analysis identifies a susceptibility locus for HIV-1. PLoS Biol 6: e32.
63. Oswald-Richter K, Grill SM, Shariat N, Leelawong M, Sander MA, et al. (2004) HIV Infection of Naturally Occurring and Genetically Reprogrammed Human Regulatory T-cells. PLoS Biol 2: E198.

64. Kinter AL, Hennessey M, Bell A, Kern S, Lin Y, et al. (2004) CD25+ regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4+ and CD8+ HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. J Exp Med 200: 331–343.

65. Sereti I, Imamichi H, Natarajan V, Imamichi T, Ramchandani MS, et al. (2005) STAT5-signaling cytokines regulate the expression of FOXP3 in CD4+ regulatory T cells. Eur J Immunol 35: 1681–1691.

66. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, et al. (2007) Activation-induced FOXP3 expression in human T effector cells does not suppress proliferation or cytokine production. Int Immunol 19: 345–354.

67. Allan SE, Crome SQ, Crollin NK, Passerini L, Steiner TS, et al. (2007) Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med 203: 1693–1700.

68. Morgan ME, van Biljon JH, Bakker AM, Heenemker B, Schilham MW, et al. (2005) Expression of FOXP3 mRNA is not confined to CD4+CD25+ regulatory T cells in humans. Hum Immunol 66: 13–20.

69. Passerini L, Allan SE, Battaglia M, Di Nunzio S, Ahtad AN, et al. (2008) STAT3-signaling cytokines regulate the expression of FOXP3 in CD4+CD25+ regulatory T cells and CD4+CD25+ effector T cells. Int Immunol 20: 421–431.

70. Roncarolo GM, Brown PJ, Maestre L, Hue S, Martinez-Torrecuadrada JL, et al. (2005) Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. Eur J Immunol 35: 1681–1691.

71. Tran DQ, Ramsey H, Shevach EM. (2007) Comparison of CD4+ T Cells Specific for HIV, HCV and CMV Revealed in Frequency, Immunodominance, Phenotype, and IL-2 Responsiveness. J Virol 82: 1246–1254.

72. Morgan ME, van Biljon JH, Bakker AM, Heenemker B, Schilham MW, et al. (2005) FOXP3 expression inversely correlates with FoxP3 and suppressive function of human regulatory T cells in all populations of HIV-infected persons. J Infect Dis 201: 331–335.

73. Sereti I, Imamichi H, Natarajan V, Imamichi T, Ramchandani MS, et al. (2005) FOXP3 T cells in all populations of HIV-infected persons. J Infect Dis 201: 331–335.

74. Lee MR, Putnam AL, Xu-Yu Z, Szot GL, Liu W, et al. (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human regulatory T cells. J Exp Med 203: 1701–1711.

75. Hartigan-O’Connor DJ, Poen C, Sinclair E, McCune JM. (2007) Human CD4+ regulatory T cells express lower levels of the IL-7 receptor alpha chain (CD127), allowing consistent identification and sorting of live cells. J Immunol Methods 319: 41–52.

76. Del Pozo-Balado Mdel M, Leal M, Mendez-Lagares G, Pacheco YM. CD14+CD25+/-hi/CD127lo phenotype does not accurately identify regulatory T cells in all populations of HIV-infected persons. J Infect Dis 201: 331–335.

77. Owen RE, Heitman JW, Hirschhorn DF, Lanteri MC, Biwas HH, et al. HIV+ elite controllers have low HIV-specific T-cell activation yet maintain strong, polfunctional T-cell responses. AIDS 24: 1095–1105.

78. Para MF, Khalil LA, Collier AC, Pollard RB, Kumar PN, et al. (2001) Qualitative and quantitative PCR measures of cytomegalovirus in patients with advanced HIV infection who require transfusions. J Acquir Immune Defic Syndr 26: 320–325.

79. Anthony DD, Younger NA, Post AB, Asaad R, Heizmann CP, et al. (2004) Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection. J Immunol 172: 4907–4916.

80. Janssen RS, Satten GA, Stramer SL, Rawal BD, O’Brien TR, et al. (1998) New seroconversion following nonoccupational postexposure prophylaxis against HIV. Clin Infect Dis 31: 1307–1313.

81. Sajadi MM, Shakeri N, Taiwani R, Redfield RR. Hepatitis C infection in HIV-1 natural viral suppressors. AIDS 24: 1669–1693.

82. Hsue PY, Hunt PW, Sinclair E, Breil B, Franklin A, et al. (2006) Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses. AIDS 20: 2275–2283.

83. Roland ME, Neilsans TB, Korne MR, Katz MH, Frances K, et al. (2005) Seroconversion following nonoccupational postexposure prophylaxis against HIV. Clin Infect Dis 41: 1307–1313.

84. Janssen RS, Satten GA, Stramer SL, Rawal BD, O’Brien TR, et al. (2006) Seroconversion following nonoccupational postexposure prophylaxis against HIV. Clin Infect Dis 41: 1307–1313.

85. Deeks SG, Martin MN, Sinclair E, Harris J, Neilsans TB, et al. (2004) Strong cell-mediated immune responses are associated with the maintenance of low-level viremia in antiretroviral-treated individuals with drug-resistant human immunodeficiency virus type 1. J Infect Dis 189: 312–321.

86. Hatano H, Delwart EL, Norris PJ, Dunn-Williams J, et al. (2009) Evidence for persistent low-level viremia in individuals who control human immunodeficiency virus in the absence of antiretroviral therapy. J Virol 83: 329–335.

87. Roland ME, Neilsans TB, Korne MR, Katz MH, Frances K, et al. (2005) Seroconversion following nonoccupational postexposure prophylaxis against HIV. Clin Infect Dis 41: 1307–1313.

88. Davidson R, MacKinnon J. (1993) Estimation and Inference in Econometrics. New York: Oxford University Press.