Spontaneous Control of HIV Replication, but not HAART-Induced Viral Suppression, Is Associated With Lower Activation of Immune Cells

Natalia A. Taborda, MSc, María T. Rugeles, MSc, DSc, and Carlos J. Montoya, MD, MSc, DSc

Abstract: HIV replication control is important to reduce AIDS progression. We determined frequency and activation status of immune cells in spontaneous HIV controllers vs. individuals with highly active antiretroviral therapy (HAART)-controlled viral load. HIV controllers exhibited significantly higher frequency of $CD4^+$ T cells and myeloid dendritic cells compared with HAART-controlled viral load. Additionally, HIV controllers have a significantly lower percentage of cells expressing activation markers on $CD4^+$ and $CD8^+$ T cells, myeloid dendritic cells, and natural killer cells. These findings suggest that during HIV infection, conservation of a normal frequency and physiological range of immune activation is associated with spontaneous, but not HAART-induced, control of viral replication.

Key Words: HIV, viral replication, HIV controllers, immune activation, immune preservation

(*J Acquir Immune Defic Syndr* 2014;66:365–369)

INTRODUCTION

HIV infection is a chronic disease characterized by persistent immune activation and inflammatory responses, leading to dysfunction and depletion of several leukocyte subpopulations, in particular $CD4^+$ T cells. Although HIV infection is associated with induction of strong immune responses, in most individuals, long-term control of HIV replication is limited.[1,2]

There are 2 new subgroups of HIV-positive individuals who exhibit a spontaneous and sustained control of viral replication, at least for 1 year, without antiretrovirals; the first group, known as elite controllers, maintain undetectable HIV RNA levels (<50 copies/mL of plasma), and the second group, known as viremic controllers, maintain detectable but low viral loads (VLs) (<2000 copies/mL).[3] They have revealed the existence of natural resistance against HIV progression, underlying the importance of defining mechanisms associated with this viral control, as a crucial step for the development of new therapeutic and vaccination strategies, currently defined as functional cure of HIV infection.[4] In most HIV controllers, an effective immune response seems to be the key to inhibit HIV replication rather than being infected with defective virus or having target cells with low ability to replicate the virus.[5,6] This effective immune response against HIV could impact also the immune activation, the main mechanism of cell death during the chronic phase.

In infected individuals, the early establishment of highly active antiretroviral therapy (HAART) contributes to a better prognosis of HIV infection.[7] However, in developing countries, most HIV infections are diagnosed in the AIDS phase, and in those diagnosed in earlier phases, treatment institution could be delayed because of socioeconomical issues. All these factors are associated with decreased efficiency of antiretroviral therapies and incomplete restoration of the immune system. However, an early and spontaneous immune response in HIV controllers can reduce the effects of viral replication, including lower immune activation, and preserving the immune response.

We hypothesize that spontaneous, but not HAART-induced, control of HIV replication is associated with higher frequency and lower activation of immune cells, preserving their functional activity. This study evaluated quantitative and qualitative parameters of T lymphocytes, myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs), invariant natural killer T (iNKT) cells, and natural killer (NK) cells, and their association with the spontaneous vs. HAART-induced viral control, in the following individuals: 39 chronically HIV-infected individuals with HAART-controlled VL and 16 HIV controllers.

MATERIALS AND METHODS

Study Population

HIV-infected individuals were recruited from health programs in Medellín, Colombia. Individuals were classified as follows: (1) HAART-naive patients with spontaneous control of viral replication (designated as HIV controllers, n = 16) and (2) patients on HAART with undetectable VL (designated HAART-controlled VL, n = 39). For HIV controllers, as was described previously,[3] the diagnosis was confirmed at least 1 year before the enrollment (mean time of diagnosis: 35 months, range: 12–168 months), and they exhibited a spontaneous and stable plasma VL below 2000 copies per milliliter

Received for publication April 3, 2014; accepted April 3, 2014.

From the Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia UdeA, Medellín, Colombia.

Supported by CODI-Universidad de Antioquia Convocatoria Mediana Cuantía 2011; Estrategia para Sostenibilidad de Grupos 2013–2014 Universidad de Antioquia; N.A.T. received a scholarship from Colciencias "Convocatoria Nacional 511 para el estudio de Doctorado en Colombia año 2010."

The authors have no conflicts of interest to disclose.

Correspondence to: María T. Rugeles, MSc, DSc, Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia (e-mail: mtrugel@catios.udea.edu.co).

during the last year (mean: 800 copies/mL, range min–max: 20–1464 copies/mL). For the selection of HAART-controlled VL group, individuals on antiretroviral therapy were followed up for VL and CD4 counts every 6 months; they had a nadir CD4 count of 214 cells per microliter (range min–max: 70–310 cells/μL); 82% started first-line HAART, at least 1 year before enrollment and before AIDS development. From the total of HAART-treated patients that we followed up, 39 reached stable control of VL (<50 copies/mL) and were included in the study.

All patients included were negative for hepatitis B and C. Four individuals from HAART-controlled VL group were diagnosed with tuberculosis before HAART, but it was efficiently treated without reactivation. None of the HIV controllers had clinical or laboratory-diagnosed tuberculosis. HIV controllers were homozygous wild-type for CCR5, whereas 2 patients from the HAARTcontrolled VL group were heterozygous for the CCR5-Delta32 allele.

This study was approved by the University of Antioquia Ethical Review Board. Clear explanation of the objectives and implications of results were given; subsequently, an institution-approved informed consent was signed.

Viral Load

The plasma VL was determined using the commercial assay reverse transcriptase–polymerase chain reaction Ampliprep-Cobas Amplicor (Roche, Indianapolis, IN), following the protocol of manufacturer; this test has a detection limit of 50 copies per milliliter.

Reagents

The following fluorochrome-labeled mouse monoclonal antibodies were from Becton Dickinson (BD, San Jose, CA): lineage marker (Lin-1, a mixture of anti-CD3, CD14, CD16, CD19, CD20, and CD56), CD8, CD11c, CD86, and CD123; and from eBioscience (San Diego, CA): anti-CD3, CD4, CD16, CD38, CD56, CD69 and HLA-DR and the CDR3 region of the invariant TCR α chain (clone 6B11).

Flow Cytometry

Frequency and phenotype of the subpopulations of leukocytes in blood were determined by flow cytometry. Briefly, 150 μL of EDTA-anticoagulated peripheral blood (PB) were incubated with specific monoclonal antibodies for 25 minutes at room temperature in darkness. Erythrocytes were lysed by incubating for 10 minutes with 2 mL of lysing solution (BD); cells were then washed twice with 2 mL of cold phosphate-buffered saline at 250*g* for 5 minutes and fixed with 250 mL of 2% paraformaldehyde.

The gate of lymphocytes was used to analyze the following populations: NK cells ($CD3^-/CD16^+/CD56^+$), iNKT cells ($6B11^+/CD3^+$), and T lymphocytes ($CD3^+/CD4^+$ and $CD3^+/CD8^+$). In the gate of mononuclear cells (peripheral blood mononuclear cell), the mDCs were determined as $Lin^-/HLA\text{-}DR^+/CD11c^+$, whereas the pDCs were defined as $Lin^-/HLA\text{-}DR^+/CD123^+$. The level of immune activation was determined by the co-expression of CD38 and HLA-DR on $CD4^+$ and $CD8^+$ T lymphocytes, the expression of CD69 on NK cells, and the expression of CD86 on mDC and pDC.

Appropriate isotype-matched control antibodies were included. Flow cytometry was performed using the BD FACS CANTO II instrument and analyzed with the BD FACSDiva software, version 6.1.2.

Statistical Analysis

Results are presented as median and range. To compare data from HIV controllers vs. progressors, a nonparametric test (Mann–Whitney *U* test, 2-tailed test) was performed. $P < 0.05$ was considered statistically significant. The statistical tests were performed using the Graph-Pad Software version 5.00.

RESULTS

Normal Frequency of Immune Cells in Spontaneous Controllers of HIV Replication

Sixteen HIV controllers (8 men vs. 8 women; age range: 21–44 years; time of infection median: 3 years, range min–max: 1–14 years) and 39 individuals with HAART-controlled VL (31 men vs. 8 women; age range: 21–60 years; time of infection: 7 years, range min–max: 3.5–11 years) were included. We found that although both HIV controllers and individuals with HAART-controlled VL had low levels of VL at the sampling time, HIV controllers had higher $CD4^+$ T-cell counts (953, 402–1525 vs. 504, 170–1283; $P < 0.0001$). Thus, we investigated whether this finding of immune preservation, associated with spontaneous control of HIV replication, could be extended to other subpopulations of immune cells in PB.

HIV controllers exhibited a significantly lower percentage of $CD8^+$ T cells compared with individuals with HAART-controlled VL (40.5%, 21.38%–55.38% vs. 62.1%, 45%–85%; $P < 0.0001$; Fig. 1A). Regarding mDC, its percentage in PB was significantly higher in HIV controllers than HAART-controlled VL patients (0.55%, 0.11%–0.96% vs. 0.36%, 0.18%–0.87%; $P = 0.035$; Fig. 1B). In contrast, both groups of patients exhibited a similar percentage of pDCs (0.15%, 0.05%–0.31% vs. 0.13%, 0.04%–0.41%; $P = 0.73$; Fig. 1C). The percentage of circulating NK and iNKT cells was also similar among the groups evaluated: 6.85%, 3.54%–23.4% vs. 7.03%, 2.69%–19.98%, $P = 0.94$ (Fig. 1D) and 0.09%, 0.03%–0.30% vs. 0.08%, 0%–0.88%, $P = 0.29$ (Fig. 1E).

Lower Level of Immune Activation in HIV Controllers

As shown in Figures 2A and B, HIV controllers exhibited a significantly decreased percentage of $CD4^+$ and $CD8^+$ T cells co-expressing HLA-DR and CD38 when compared with HAART-treated patients (2.3%, 0.76%–5.39% vs. 5.74%, 1.6%–33.8%, $P < 0.001$ for $CD4^+$ T cells and 4.8%, 1.67%–28.14% vs. 17.32%, 1.37%–56.62%; $P = <0.0001$ for $CD8^+$ T cells).

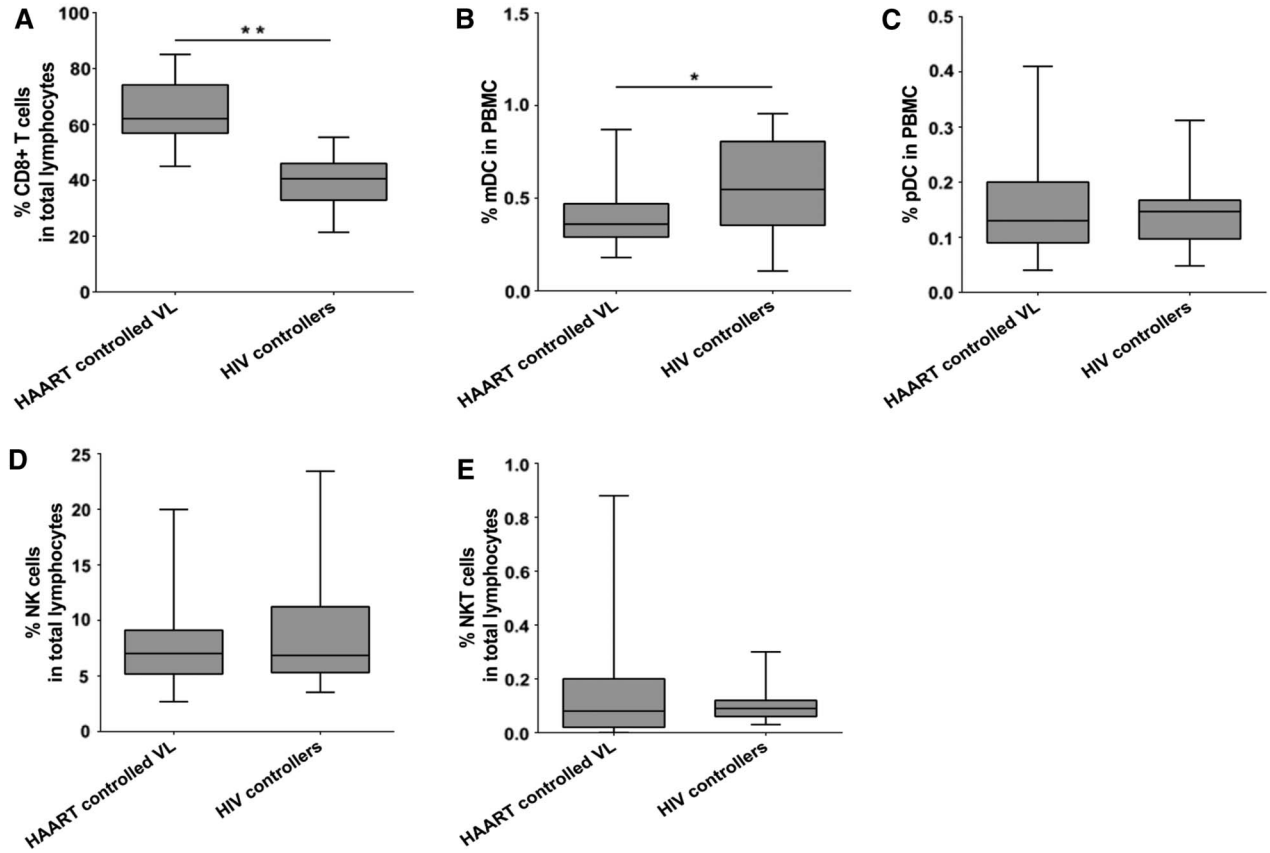

FIGURE 1. Quantitative evaluation of innate and adaptive immune cells in PB of HIV-infected individuals. PB cells were incubated with monoclonal antibodies against human molecules on innate and adaptive immune cells and detected by flow cytometry as described in Materials and Methods. The figure shows percentage of CD8+ T cells (A), mDC (B), pDC (C), NK cells (D) and NKT cells (E). The results are presented as median, range minimum and maximum. Mann–Whitney *U* test was used with a confidence level of 95%. Significant differences are indicated at the top of the figure. $*P < 0.05$, $**P < 0.001$.

In addition, HIV controllers exhibited lower frequency of mDCs expressing CD86 (0.02%, 0.01%–0.48% vs. 5.15%, 0.3%–18.3%; $P < 0.0001$; Fig. 2C), whereas the frequency of pDCs expressing CD86 was similar in HIV controllers and HAART-treated patients (0.03%, 0%–2.38% vs. 1.15%, 0%–3.9%; $P = 0.80$, respectively; Fig. 2D).

Finally, the expression of CD69 by NK cells was also significantly lower in HIV controllers than in HAART-treated patients (1.53%, 0%–5.85% vs. 8%, 4.8%–12.1%; $P < 0.0001$; Fig. 2E).

DISCUSSION

A new subgroup of HIV seropositive individuals was recently described,[3] the HIV controllers, who exhibit a spontaneous and sustained control of viral replication in the absence of antiretrovirals. Previously, we reported that HIV controllers have higher frequency of mDCs, lower percentage of regulatory T cells, and lower expression of activation molecules compared with progressors. However, there are not studies defining distinct immune parameters between individuals with spontaneous vs. HAART-controlled VL to determine the implications of an early viral control. Therefore, we evaluated the frequency and immune activation of different immune cells in a cohort of HIV controllers, who were compared with HAART-treated patients. HIV controllers exhibited a higher percentage of $CD4^+$ T cells and lower $CD8^+$ T cells compared with HAART-treated patients. Moreover, HIV controllers exhibited a higher frequency of mDCs. Although we did not observe any other statistically significant difference with other immune cells, these results suggest that HIV controllers conserve a normal level of the most important immune cell populations, finding that could be associated with effective immune responses. Interestingly, the expression of activation molecules in HIV controllers was lower in $CD4^+$ and $CD8^+$ T cells, mDCs, and NK cells compared with that in HAART-controlled VL patients. In accordance with our results, previous studies documented low activation in HIV controllers.[8,9] However, other studies observed a similar or even higher expression of immune activation molecules in HIV controllers, when compared with HAART-treated patients.[10,11] These results could be explained by the fact that HIV controllers are a heterogeneous population and the mechanisms to control viral replication are not the same for all cohorts of HIV controllers. Although HAART administration is usually associated with a decrease in viral

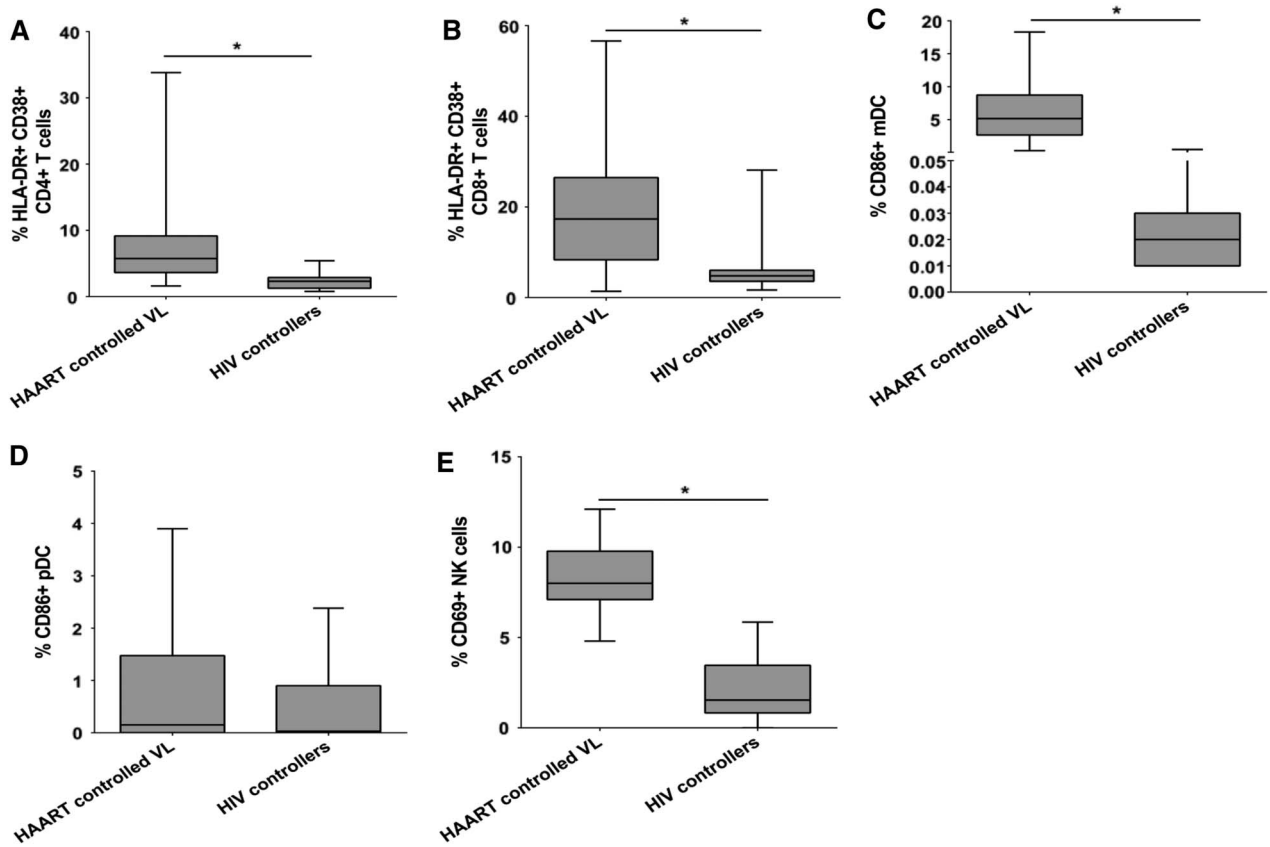

FIGURE 2. Expression of immune activation molecules on innate and adaptive cells of HIV-infected individuals. The co-expression of HLA-DR and CD38 on T cells (A and B), the expression of CD86 on mDC and pDC (C and D), and the expression of CD69 on NK cells (E) were evaluated by flow cytometry, using the fluorescence minus one method. The results are presented as median, range minimum and maximum. Mann–Whitney *U* test was used with a confidence level of 95%. Significant differences are indicated at the top of the figure. $*P < 0.001$.

replication, the immune restoration is variable depending among other factors, on the time of HAART institution. Indeed, there is a high risk of virological failure, opportunistic infections, and death in patients with late administration of HAART.[12]

HIV infection induces alterations in multiple cells and tissues; the $CD4^+$ T cells are the main target cells, particularly effector memory T cells, and therefore, $CD4^+$ T-cell count is the most relevant marker of progression. From the acute phase on, these cells decrease in PB, but mainly in the gut-associated lymphoid tissue, the most important site of viral replication.[13] As a result, there is a massive destruction of the mucosal tissue, favoring microbial translocation from the intestinal lumen to systemic circulation, inducing a chronic and persistent state of immune activation. This state is characterized by the expression of activation markers in immune cells and by high levels of proinflammatory cytokines that finally trigger the immune exhaustion phenomenon and massive activation-induced cell death.[14,15] We have demonstrated that HIV controllers exhibit low level of expression of activation markers and conserve a normal frequency of circulating immune cells, including $CD4^+$ T cells, in contrast to HAART-controlled VL patients, who exhibit most of the abnormalities so far described. Currently, we are evaluating whether this pattern of immune preservation is similar in lymphoid tissue from intestinal mucosa.

These results suggest that HAART do not reset immune activation to normal levels, indicating that this factor can limit the immune restoration. In contrast to HAART-treated patients, HIV controllers maintain an effective antiviral activity in both $CD4^+$ and $CD8^+$ T lymphocytes.[11,16] Interestingly, Th17 cells in intestinal mucosa and PB correlate with a stable and efficient control of viral replication in HIV controllers,[17,18] but not in HAART-treated patients, suggesting that therapy is not enough to restore entire immune functions. Several mechanisms have been proposed to participate in the viral control exhibited in HIV controllers, including (1) low frequency of regulatory T cells in blood and intestinal tissues that have high functional capacity associated with decreased levels of immune activation,[19] (2) increased HIV-specific IL-21^+ $CD4^+$ T-cell responses, associated with optimal cytotoxic responses mediated by $CD8^+$ T cells,[20] (3) greater cytolytic activity of HIV-specific $CD8^+$ T cells,[21] mainly directed to cells expressing different Gag epitopes, (4) higher HIV-specific neutralizing antibody responses,[22] and (5) increased expression of soluble antiviral proteins.[15] Because we did not evaluate any of these mechanisms, some of them could be also involved in the spontaneous control of viral replication.

However, HIV controllers are a heterogeneous group, as was demonstrated in studies of genome-wide sequence where only a small fraction of spontaneous control was explained by some specific HLA alleles, suggesting other mechanisms involved in this control.[23]

The overall evaluation of different immune mechanisms associated with the spontaneous control of HIV replication could lead to a better understanding of the mechanisms involved in the natural resistance to HIV infection, eventually pointing to new therapeutic targets to control this infection.

ACKNOWLEDGMENTS

The authors thank Anne-Lise Haenni for all her constructive comments, and the CIHR IID&GH Program of the University of Manitoba.

REFERENCES

1. Giorgi JV, Liu Z, Hultin LE, et al. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr.* 1993;6:904–912.
2. Landay AL, Mackewicz CE, Levy JA. An activated CD8+ T cell phenotype correlates with anti-HIV activity and asymptomatic clinical status. *Clin Immunol Immunopathol.* 1993;69:106–116.
3. Walker BD. Elite control of HIV infection: implications for vaccines and treatment. *Top HIV Med.* 2007;15:134–136.
4. Vanham G, Van Gulck E. Can immunotherapy be useful as a "functional cure" for infection with human immunodeficiency virus-1? *Retrovirology*. 2012;9:72.
5. Blankson JN, Bailey JR, Thayil S, et al. Isolation and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. *J Virol.* 2007;81:2508–2518.
6. Julg B, Pereyra F, Buzón MJ, et al. Infrequent recovery of HIV from but robust exogenous infection of activated CD4(+) T cells in HIV elite controllers. *Clin Infect Dis.* 2010;51:233–238.
7. Cellerai C, Harari A, Stauss H, et al. Early and prolonged antiretroviral therapy is associated with an HIV-1-specific T-cell profile comparable to that of long-term non-progressors. *PLoS One.* 2011;6:e18164.
8. Shaw JM, Hunt PW, Critchfield JW, et al. Increased frequency of regulatory T cells accompanies increased immune activation in rectal mucosae of HIV-positive noncontrollers. *J Virol.* 2011;85:11422–11434.
9. Card CM, Keynan Y, Lajoie J, et al. HIV controllers are distinguished by chemokine expression profile and HIV-specific T-cell proliferative potential. *J Acquir Immune Defic Syndr.* 2012;59:427–437.
10. Hunt PW, Brenchley J, Sinclair E, et al. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *J Infect Dis.* 2012;197:126–133.
11. Vingert B, Benati D, Lambotte O, et al. HIV controllers maintain a population of highly efficient Th1 effector cells in contrast to patients treated in the long term. *J Virol.* 2012;86:10661–10674.
12. Pérez-Molina JA, Suárez-Lozano I, Del Arco A, et al. Late initiation of HAART among HIV-infected patients in Spain is frequent and related to a higher rate of virological failure but not to immigrant status. *HIV Clin Trials.* 2011;12:1–8.
13. Brenchley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? *Nat Immunol.* 2006;7:235–239.
14. Shebl FM, Yu K, Landgren O, et al. Increased levels of circulating cytokines with HIV-related immunosuppression. *AIDS Res Hum Retroviruses.* 2011;8:809–815.
15. Taborda NA, Cataño JC, Delgado JC, et al. Higher SLPI expression, lower immune activation, and increased frequency of immune cells in a cohort of Colombian HIV-1 controllers. *J Acquir Immune Defic Syndr.* 2012;60:12–19.
16. Hersperger AR, Martin JN, Shin LY, et al. Increased HIV-specific CD8+ T-cell cytotoxic potential in HIV elite controllers is associated with T-bet expression. *Blood.* 2011;117:3799–3808.
17. Salgado M, Rallón NI, Rodés B, et al. Long-term non-progressors display a greater number of Th17 cells than HIV-infected typical progressors. *Clin Immunol.* 2011;139:110–114.
18. Ciccone EJ, Greenwald JH, Lee PI, et al. CD4+ T cells, including Th17 and cycling subsets, are intact in the gut mucosa of HIV-1-infected long-term nonprogressors. *J Virol.* 2011;85:5880–5888.
19. Angin M, Kwon DS, Streeck H, et al. Preserved function of regulatory T cells in chronic HIV-1 infection despite decreased numbers in blood and tissue. *J Infect Dis.* 2012;205:1495–1500.
20. Williams LD, Bansal A, Sabbaj S, et al. Interleukin-21-producing HIV-1-specific CD8 T cells are preferentially seen in elite controllers. *J Virol.* 2011;85:2316–2324.
21. Hersperger AR, Pereyra F, Nason M, et al. Perforin expression directly ex vivo by HIV-specific CD8 T-cells is a correlate of HIV elite control. *PLoS Pathog.* 2010;6:e1000917.
22. Pereyra F, Palmer S, Miura T, et al. Persistent low-level viremia in HIV-1 elite controllers and relationship to immunologic parameters. *J Infect Dis.* 2009;200:984–990.
23. Limou S, Zagury JF. Immunogenetics: genome-wide association of non-progressive HIV and viral load control: HLA genes and beyond. *Front Immunol.* 2013;4:118.