Higher SLPI Expression, Lower Immune Activation, and Increased Frequency of Immune Cells in a Cohort of Colombian HIV-1 Controllers

Natalia A. Taborda, BS, MS, Juan C. Cataño, MD,† Julio C. Delgado, MD, MS,‡ María T. Rugeles, BS, MS, PhD,* and Carlos J. Montoya, MD, MS, PhD**

Background: There are 2 new phenotypes of HIV-1–positive individuals who exhibit a spontaneous and sustained control of viral replication at least for 1 year without antiretroviral therapy (elite controllers <50 copies/mL and viremic controllers <2000 copies/mL). Mechanisms related to this spontaneous control of viral replication are poorly understood.

Methods: The study included HIV-1 controllers (patients with at least 1 year of HIV-1 diagnosis, highly active antiretroviral therapy naive, and with viral loads less than 2000 copies/mL) and HIV-1 progressors without antiretroviral therapy (viral load >2500 copies/mL, and CD4$^+$ T-cell count >250 cells/μL at the time of sampling). The expression of soluble factors, leukocyte protease inhibitor (SLPI) and human α-defensins-1 (HAD-1), was measured by real-time polymerase chain reaction from neutrophil cultures with or without HIV stimulation; the frequency and phenotype of innate and adaptive immune cells were determined by flow cytometry, and frequency of human leukocyte antigen alleles was determined by polymerase chain reaction sequence–specific oligonucleotide typing.

Results: As expected, HIV-1 controllers had higher CD4$^+$ T-cell counts and lower viral load when compared with HIV-1 progressor individuals; in addition, they exhibited lower expression of activation markers, higher frequency of myeloid dendritic cell, lower percentage of regulatory T cells and natural killer cells, and higher expression of SLPI.

Conclusions: All together, these findings suggest that the control of the immune activation status and the production of antiviral proteins by innate immune cells could be associated to the mechanisms involved in the control of HIV-1 replication and better preservation of the CD4 T-cell count.

Key Words: HIV-1, regulatory T cells, cellular immune response, disease progression, human SLPI protein

(*J Acquir Immune Defic Syndr* 2012;60:12–19)

INTRODUCTION

HIV-1 infection induces alterations in multiple components of the immune system, reflected mainly by a decrease in the CD4$^+$ T-cell population, leading to AIDS. However, the natural history of HIV-1 infection is heterogeneous, given the variability in the time required to advance to AIDS. In most HIV-1–infected individuals, the median time is 8–10 years[1]; nonetheless, a small fraction of HIV-1–infected individuals who despite being infected for more than 10 years remain symptom less, maintaining normal peripheral blood (PB) CD4$^+$ T-cell counts (>500 CD4$^+$ cells/μL), in the absence of antiretroviral therapy; they are known as long-term nonprogressors (LTNPs).[2,3] In addition, there are 2 new subgroups of HIV-1–positive individuals who exhibit a spontaneous and sustained control of viral replication, at least for 1 year without antiretroviral therapy: elite controllers maintain undetectable HIV-1 RNA levels (<50 copies/mL), and viremic controllers exhibit detectable but low viral loads (<2000 copies/mL).[4] These subgroups have revealed the existence of natural mechanisms of viral control.

Several studies have aimed at determining the mechanisms involved in HIV-1 resistance and viral control; so far, resistance has been associated with host genetic variants and immunological factors that limit viral infection and progression.[5] The CCR5-delta 32 mutation has been the most widely investigated, and its association with HIV-1 resistance relies on the inability of R5 viral strains to enter target cells. Other single-nucleotide polymorphisms in HIV-1 coreceptors and their ligands are associated with this phenomenon.[6] Also, several reports on cellular immunity indicate that apoptosis of target cells,[7] increased production of interferon-γ by natural

Received for publication June 1, 2011; accepted December 27, 2011.

From the *Immunovirology Group, School of Medicine, Universidad de Antioquia, Medellin, Colombia; †Section of Infectious Diseases, Department of Internal Medicine, Medical School, Universidad de Antioquia, Medellin, Colombia; and ‡ARUP Institute for Clinical and Experimental Pathology, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Supported by "Sostenibilidad 20011-2012" from the University of Antioquia, Banco de la República, COLCIENCIAS (111540820498 to M.T.R. and "Convocatoria Nacional 511 para el estudio de Doctorado en Colombia año 2010").

Presented at Keystone Symposia on Molecular and Cellular Biology, March 2011, Whistler, Canada.

N. A. Taborda received a scholarship from Colciencias; M. T. Rugeles received a grant (111540820498) from Colciencias and Sostenibilidad 2011-2012 from the Universidad de Antioquia, Colombia. All other authors have no conflicts of interest to disclose.

All other authors have no conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

Correspondence to: Carlos J. Montoya, MD, MS, PhD, Immunovirology Group, School of Medicine, Universidad de Antioquia, Calle 62, # 52-59, Torre 2, Laboratory 532, Medellin, Colombia (e-mail: cjmonto@gmail.com).

killer (NK) cells,[8] increased expression of NK receptors associated with cellular activity,[9,10] and certain human leukocyte antigen (HLA) alleles[11,12] are other factors associated with HIV-1 resistance. Finally, humoral response also contributes to HIV-1 resistance; in fact, a higher production of mucosal anti-HIV-1 IgA has been reported in exposed seronegatives (ESNs) compared with healthy controls.[13]

Regarding soluble factors, RNases, chemokines, and cationic proteins have been associated with HIV-1 protection in ESNs or LTNPs.[14–17] The secretion of leukocyte protease inhibitor (SLPI) is induced by HIV-1 gp120 in oral epithelial cells without requiring infection.[18] In addition, McNeely et al[19,20] showed that SLPI protects against HIV-1 infection, an inhibitory effect that occurs before reverse transcription. Also, a higher production of human α-defensins (HAD-1 to -3) has been associated with inhibition of HIV-1 replication.[21,22]

However, it is currently accepted that one of the most important pathogenic mechanism during chronic HIV-1 infection that has been correlated to AIDS progression is the hyperactivation of immune cells. Abnormal activation is induced by exposure to viral antigens and then exacerbated by destruction of mucosal lymphoid tissue,[23] leading to the translocation of bacteria from the intestinal lumen to systemic circulation.[24] As a result, there is an increased number of activated target cells for HIV-1,[25] an increased apoptosis of activated $CD4^+$ T cells, anergy, and, in general, a functional alteration of the immune system. As a counteracting mechanism, regulatory T cells (Tregs) control cellular activation levels. However, the potential effect of Tregs on HIV-1 pathogenesis is controversial because several reports suggest that these cells might have a dual role. Their benefic potential comes from their ability to decrease cellular activation, limiting viral replication and new cell targets; in contrast, their accumulation might restrict the specific anti-HIV-1 responses, limiting clearance of infected cells.[26]

Considering that HIV-1 controllers were recently described and the mechanisms related to viral control are poorly studied, we evaluated the frequency and immune activation phenotype of immune cells and class I *HLA* alleles and the expression of the soluble factors HAD-1 and SLPI, in a Colombian cohort of HIV controllers, trying to determine their association to viral control.

METHODS

Population and Samples

Two groups were included: (1) 11 HIV-1 controllers, defined as previously described[4]; briefly, we selected HIV-infected individuals who exhibit a spontaneous and sustained control of viral replication, at least for 1 year without antiretroviral therapy: elite controllers maintain undetectable HIV-1 RNA levels (<50 copies/mL), and viremic controllers exhibit detectable but low viral loads (<2000 copies/mL); all these 11 individuals were tested negative for alleles with the delta 32 mutation in the *CCR5* gene; and (2) 11 chronic HIV-1–infected progressors, who were individuals with a $CD4^+$ T-cell count higher than 250 cells per microliter, HIV viral load higher than 2500 RNA copies per milliliter, and without highly active antiretroviral therapy at the time of sampling.

All individuals signed an informed consent approved by the Bioethical Board from Universidad de Antioquia, prepared according to Colombian Legislation, Resolution 008430 of 1993. This investigation was approved by the Ethical Committee of Universidad de Antioquia (certificate of approval 10-8-298). Twenty milliliters of PB in EDTA or heparin tubes were collected to determine the frequency and phenotype of immune cells and for cell culture, respectively.

Culture of PB Polymorphonuclear Cells

Because HAD-1 and SLPI are produced mainly by neutrophils, this cell subpopulation was isolated and cultured. Briefly, 12 mL of PB was mixed with 6 mL of 6% dextran and 0.9% NaCl; this mixture was incubated for 45 minutes at room temperature (RT), the erythrocytes were lysed with 6 mL of distillated water for 35 seconds, and the osmolarity was restored with 0.6 M KCl. Then, polymorphonuclear cells (PMNs) were isolated by density-gradient centrifugation at 700*g* for 30 minutes with Ficoll-Hypaque 1077 (Sigma, St Louis, MO). In 24-well plates, 2×10^6 PMNs were incubated in RPMI (Invitrogen, Carlsbad, CA) with 3000 pg/mL of p24 antigen, obtained from H9/HTLV-III cell supernatants (National Institutes of Health AIDS Research & Reference Reagent Program, Bethesda, MD). As a positive control, 10 μg/mL of lipopolysaccharide (LPS; Sigma) was used. For viral or LPS stimulation, the cells were incubated for 2 hours at 37°C in 5% CO_2 and then washed and re-incubated for an additional 48 hours at 37°C in 5% CO_2. Finally, PMNs were collected for RNA extraction.

Real-Time Reverse Transcriptase–Polymerase Chain Reaction Assay to Quantify HAD-1 and SLPI mRNA

RNA was extracted using TRizol Reagent (Invitrogen) following the manufacturer's instructions. The amount and purity of the RNA were determined by spectrometry at 260/280 nm. Total RNA was treated with DNase I (Fermentas, St Leon-Rot, Germany). DNA was synthesized using SuperScript III (Invitrogen), according to the manufacturer's instructions.

Each 20 μL of real-time polymerase chain reaction (PCR) mixture consisted of 2 μL of cDNA, 10 μL of Maxima SYBR Green/qPCR Master Mix (Fermentas), and primers (0.4 μM each). The primer sequences and product size for *HAD-1* and *SLPI* were previously reported[27,28]; the sequences and cycling profiles are found in **Supplemental Digital Content 1** (see **Table,** http://links.lww.com/QAI/A260). Ubiquitin RNA was used to normalize the RNA content of *HAD-1* and *SLPI*. We included a melting curve to confirm the specificity of the PCR product. All real-time reverse transcriptase–PCR amplifications were performed using the CFX96 real-time system and data analysis using the software CFX Manager Version 1.5.534.0511 (Bio-Rad, Hercules, CA).

HLA Typing

HLA typing was performed by the Histocompatibility and Immunogenetics Clinical Laboratory of University of Utah. The determination of the *HLA-A* and *HLA-B* alleles was performed by a DNA hybridization assay (One-Lambda, Canoga Park, CA) using multicolored beads with unique flurophores bound to amplified PCR products. Detection was via a Luminex 100 Flow cytometer.

Flow Cytometry

Flurochrome-labeled mouse monoclonal antibodies against human molecules were from Becton Dickinson (BD, San Jose, CA): CD8, CD86, lineage marker (Lin-1, a mixture of anti-CD3, CD14, CD16, CD19, CD20, and CD56), CD11c, CD123, and the CDR3 region of the invariant T-cell receptor-α chain (clone 6B11); and from eBioscience (San Diego, CA): CD3, CD4, CD16, CD25, CD38, CD56, CD69, CD127, HLA-DR, and FoxP3.

The frequency and phenotype of different subpopulations in PB were determined by flow cytometry. Briefly, 150 μL of PB was incubated with specific monoclonal antibodies for 25 minutes at RT in the dark. Erythrocytes were lysed by incubating for 10 minutes with 2 mL of 1× fluorescence-activated cell sorting lysing solution (BD); the cells were washed twice with 2 mL of cold phosphate-buffered saline at 250*g* for 5 minutes and fixed with 250 mL of 2% paraformaldehyde.

Lymphocyte's gate, identified by side (SSC) vs. forward (FSC) light scatter, was used to analyze the following cell populations: NK cells ($CD3^-/CD16^+/CD56^+$), invariant natural killer T cells (iNKT; $6B11^+/CD3^+$), and T cells ($CD3^+/CD4^+$ or $CD3^+/CD8^+$). The CD4/CD8 lymphocyte ratio was determined based on the percentage of $CD4^+$ and $CD8^+$ T cells. In mononuclear cells' gate, identified by SSC vs. FSC, the dendritic cells were gated as $Lin^-/HLA\text{-}DR^+$ and then as $CD11c^+$ myeloid dendritic cell (mDC) or $CD123^+$ plasmacytoid dendritic cell (pDC).

The frequency of Tregs (defined in lymphocyte's gate as $CD3^+/CD4^+/FoxP3^+/CD25^+/CD127^{low/-}$) was determined by intracellular flow cytometry. After staining with extracellular markers, the cells were permeabilized and fixed using the anti-human FoxP3 staining set (eBioscience), following manufacturer's instructions. Seven microliters of anti-FoxP3 was then added; the cells were incubated for 25 minutes at 4°C, washed twice with 2 mL of phosphate-buffered saline at 250*g* for 5 minutes, and fixed with 250 μL of 2% paraformaldehyde.

All preparations were stored at 4°C until acquisition in the cytometer FACS CANTO-II (BD). Acquisition analysis was performed in the BD FACSDiva 6.1.2 version. The analysis of activation molecules was made using the fluorescence minus one method. Dead cells were excluded from the analysis through SSC vs. FSC. Absolute numbers of different leukocyte subpopulations were calculated based on manually determined total and differential PB cell counts.

Data Analysis

To compare data from HIV-1 controllers vs. progressors, a nonparametric test (Mann–Whitney *U*–2-tailed test) was performed. $P < 0.05$ was considered statistically significant. Correlation analyses were based on Spearman correlation coefficient calculations.

Allelic distribution and frequencies of the haplotypes were determined using the software Genepop 4.0.10, and to evaluate statistically significant differences, Fisher exact test was used. Statistical tests were performed using Graph-Pad Software version 5.00.

RESULTS

HIV-1 Controllers Exhibit Differences in Immune Cells

Demographics and virological and immunological data at the time of sampling are shown in Table 1. Two elite and 9 viremic controllers were included. There were no differences in age and time length after HIV-1 diagnosis in both infected groups; as expected, HIV-1 controllers exhibited significantly lower viral load and higher $CD4^+$ T-cell count and percentage in PB when compared with progressors.

The percentage (median: 40.5%, range min-max: 22.3%–49.3%; vs. 45.2%, 29.5%–68.7; $P = 0.1679$; Fig. 1A) and absolute numbers (913.3, 564.4–2205 vs. 1168, 740–2776; $P = 0.3579$) of $CD8^+$ T cells were similar in controllers and progressors, respectively. As expected, HIV-1 progressors

TABLE 1. Demographic and Clinical Information

	HIV-1–Infected Individuals		
	Controllers (n = 11)	Progressors (n = 11)	*P*
Age, median (range), yr	29 (23–44)	33 (23–49)	0.8952
Gender, male:female	7:4	9:2	N/A
Time of diagnosis, median (range), mo	34 (13–87)	13 (1–43)	0.0850
Plasma HIV-1 viral load, median (range), RNA copies/mL	907 (40–1370)	197,500 (2770–437,000)	<0.0001
$CD4^+$ T-cell count, median (range)	952.5 (402–1525)	506.6 (294.4–768.3)	0.0031
$CD4^+$ T-cell percentage, median (range)	36 (21.8–50.9)	19.2 (5.9–29)	0.0013

N/A, not applicable.

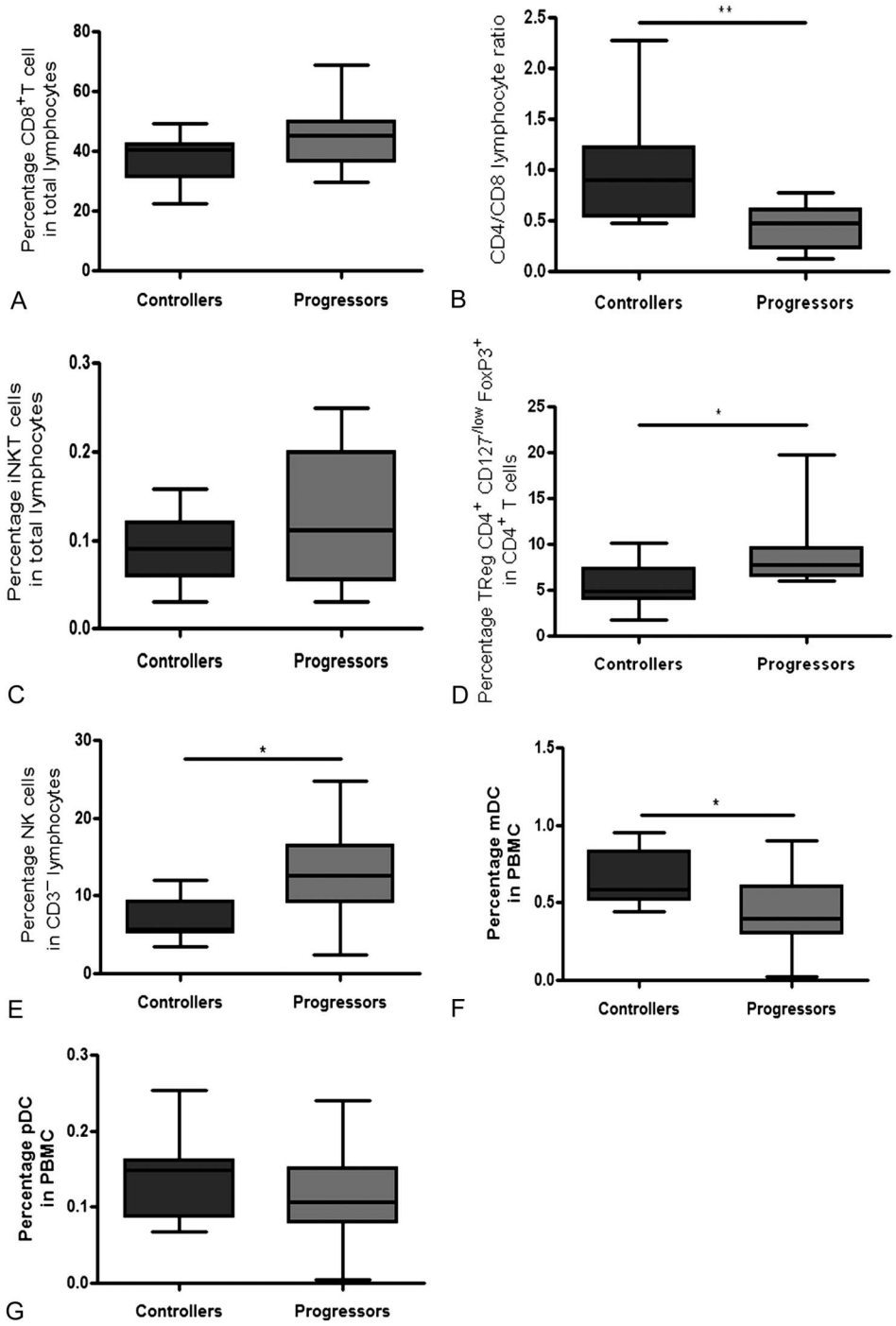

FIGURE 1. A-G.Quantitative evaluation of innate and adaptive immune cells in PB of HIV-1–infected individuals. PB cells were incubated with monoclonal antibodies against human molecules on innate and adaptive immune cells and detected by flow cytometry as described in Materials and Methods. The results are presented as median, 25% and 75% percentiles, and range. A statistical comparison between groups was performed using a Mann–Whitney *U* test with a confidence level of 95%. Significant differences are indicated at the top of the figure (*$P < 0.05$, **$P < 0.01$).

exhibited a significant decrease in the $CD4^+/CD8^+$ lymphocyte ratio when compared with controllers (0.48, 0.13–0.78 vs. 0.9, 0.5–2.3; $P = 0.0058$; Fig. 1B).

We did not find significant differences in the frequency of iNKT cells between controllers and progressors (0.09%, 0.03%–0.16% vs. 0.11%, 0.03%–0.25, respectively; $P = 0.5543$; Fig. 1C). However, the frequency of Tregs, defined as $CD4^+/FoxP3^+/CD127^{-/low}$ cells, was significantly lower in controllers when compared with progressors (4.95%, 1.8%–10.1% vs. 7.8%, 6%–19.8%, respectively; $P = 0.0222$; Fig. 1D). Similar results were obtained when the Tregs were defined as $CD4^+/CD25^+/CD127^{-/low}$ cells.

When comparing controllers and progressors, we found a significant difference in the frequency of circulating NK cells (5.7%, 3.5%–12% vs. 12.6%, 2.4%–24.8%; $P = 0.0104$; Fig. 1E); the frequency of mDC from controllers was significantly higher when compared with progressors (0.59%, 0.44%–0.96% vs. 0.4%, 0.03%–0.9%; $P = 0.0278$;

Fig. 1F), and we did not find a significant difference in the frequency of pDC (0.15%, 0.07%–0.25% vs. 0.11%, 0.004%–0.24%; $P = 0.1779$; Fig. 1G).

Decreased Expression of Immune Activation Markers in HIV-1 Controllers

HIV-1 controllers, compared with progressors, expressed a significantly lower percentage of mDC expressing CD86 (0%, 0%–0.5% vs. 0.14%, 0%–38.1%; $P = 0.0320$; Fig. 2A) and lower mean fluorescence intensity (MFI) of this molecule on mDC (0, 0–10,680 vs. 4281, 0–20,390; $P = 0.0320$). Regarding pDC, despite a lower level of expression of CD86 on cells from controllers (Fig. 2B), no significant differences in the percentage of pDC CD86$^+$ were detected (0%, 0%–2.4% vs. 0.05%, 0%–16.8%; $P = 0.4583$; Fig. 2B) or in the MFI of CD86 (0, 0–3527 vs. 2782, 0–4567; $P = 0.2664$), between both groups of infected patients.

Regarding the activation status of T cells, controllers exhibited a significantly lower percentage of CD4$^+$ T cells co-expressing CD38/HLA-DR when compared with progressors (2.3%, 0.8%–5.4% vs. 5.7%, 3.3%–10.3%; $P < 0.0004$; Fig. 2C). There was also a negative correlation between the expression of these activation molecules and the frequency of circulating CD4$^+$ T cells in controllers (Spearman $r = -0.7$; $P = 0.0165$; Fig. 2D). In contrast, in CD8$^+$ T cells, we did not find significant differences in the expression of activation markers in controllers vs. progressors (4.6%, 1.7%–6.2% vs. 6.1%, 2.5%–15.2%; $P = 0.1309$; Fig. 2E). Finally, significant differences were found when the results of controllers were compared with progressors for the percentage of NK cells expressing CD69 (1.5%, 0%–5.9% vs. 3.74%, 0.53%–19.5%; $P = 0.0417$; Fig. 2F) but

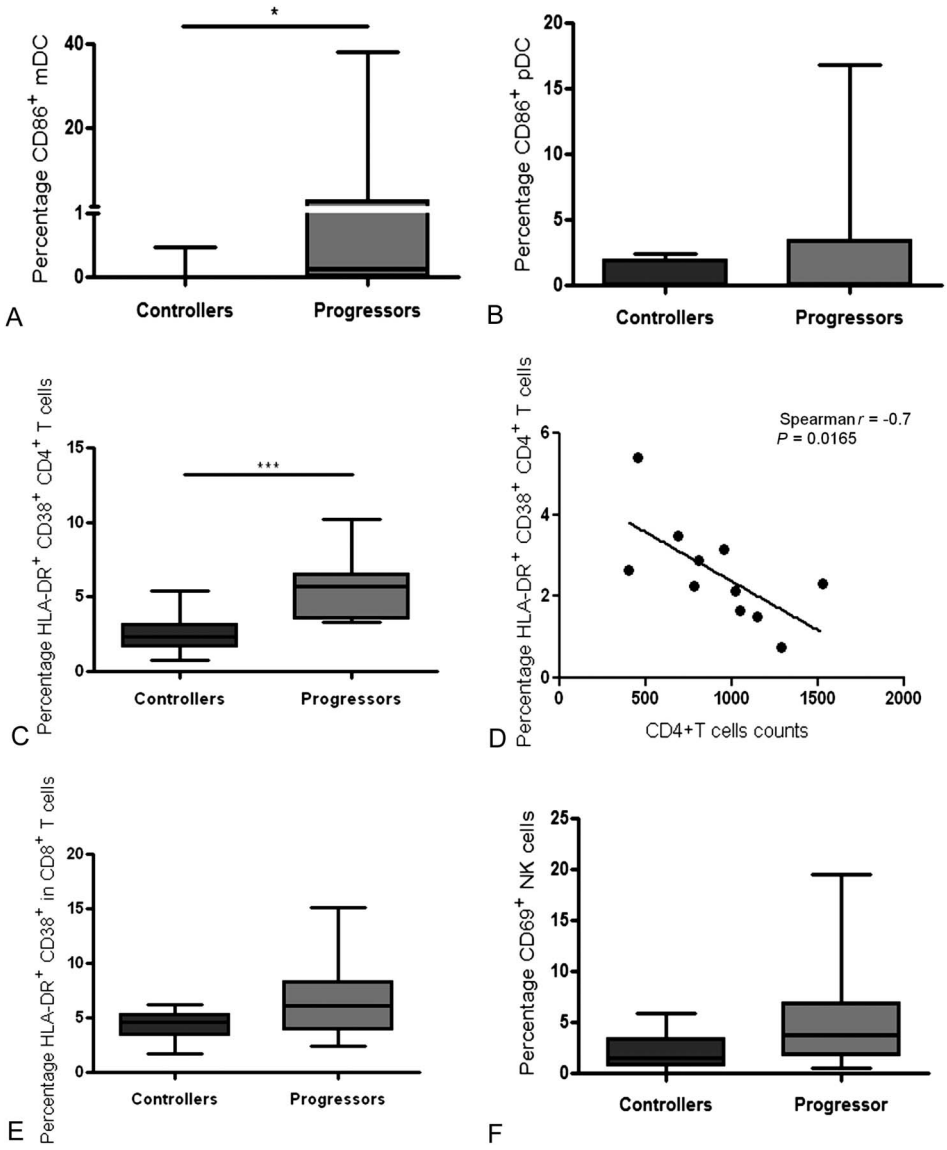

FIGURE 2. Expression of immune activation molecules on innate and adaptive cells of HIV-1–infected individuals. The expression of CD86 on DCs (A and B), the co-expression of HLA-DR and CD38 on T cells (C and E), and CD69 on NK cells (F) were evaluated by flow cytometry, using the fluorescence minus one method. D, correlation between CD4$^+$ T-cell counts and co-expression of HLA-DR and CD38 on T cells in controllers. The results are presented as median, 25% and 75% percentiles, and range. The statistical comparison between groups was performed using a Mann–Whitney *U* test with a confidence level of 95%. Significant differences are indicated at the top of the figure (*$P < 0.05$, ***$P < 0.001$).

not for the MFI of CD69 expression (1499, 0–2366 vs.1543, 925–3475; $P = 0.7927$).

PMNs From HIV-1 Controllers Express Higher SLPI

To determine if HAD-1 and SLPI, factors with anti-HIV-1 activity, were associated with the control of viral replication, we evaluated by real-time PCR the change in *HAD-1* and *SLPI* gene expression when the PMNs were stimulated with HIV-1. *SLPI* expression in controllers was significantly higher than that in progressors (11.4, −2.3 to 179.9 vs. 0, −6.4 to 9.2; $P = 0.0486$; Fig. 3A), whereas the expression of *HAD-1* was similar in both groups (0, −2.8 to 3.1 vs. −1.5, −2.3 to 13.17; $P = 0.6945$; Fig. 3B). In contrast, in LPS-stimulated PMNs, used as positive control of stimulation, no significant differences were found between controllers and progressors when the expression of *SLPI* (11.7, 3.3–40.9 vs. 11.75, 0–42.9; $P = 0.9623$) and *HAD-1* (3.3, 1–7.6 vs. 1.7, 1.39–4.9; $P = 0.1381$) was measured.

HLA Alleles

The cellular immune response of HIV-1–specific cytotoxic T lymphocytes is mediated mainly by the type of peptides displayed by class I HLA molecules. When the frequency of class I *HLA* alleles between controllers and progressors was evaluated, no association was established between the control of viral replication and the frequency of any *HLA-A* and *HLA-B* alleles (*P*-value for each allele was >0.05), previously related to resistance to HIV-1 progression (see **Tables, Supplemental Digital Content 2**, http://links.lww.com/QAI/A261).

DISCUSSION

The investigation of the mechanisms involved in natural control of HIV-1 replication is essential to develop therapeutic measures to prevent HIV-1 infection or progression. In 2007, a particular group of infected individuals was described as viral controllers; they spontaneously control HIV-1 replication, at least for 1 year, in the absence of highly active antiretroviral therapy.[4] These patients might represent a key group to study mechanisms involved in the control of viral replication. Because controllers are poorly characterized, our study focused on this population of HIV-1–infected individuals.

We evaluated the frequency and phenotype of immune cells among HIV-1–infected persons (controllers versus progressors) and investigated if the presence of particular *HLA* alleles or the levels of *SLPI* and *HAD-1* expression were associated with the viral control. Despite a longer time of HIV-1 diagnosis in HIV-1 controllers, they exhibited higher $CD4^+$ T-cell counts and lower viral load than progressors. In addition, HIV-1 controllers had a higher frequency of mDC, a lower percentage of Treg cells, and lower expression of activation molecules on $CD4^+$ T cells and mDC compared with progressors. Finally, *SLPI* expression was higher in stimulated PMN from HIV-1 controllers, compared with progressors.

SLPI is a protein previously associated with HIV-1 resistance[29–31]; higher salivary SLPI levels were associated with decreased risk of HIV-1 infection among infants exposed to HIV-1 via breastfeeding.[29] Similarly, higher SLPI levels were found in vaginal fluids of HIV-1–positive women whose babies were born uninfected than those from transmitting women[31]; these, together with our findings, suggest that a higher expression of *SLPI* is related to the control of HIV-1 transmission and replication. Previous, unpublished data from our research group indicated higher expression of SLPI in oral mucosa of ESNs compared with healthy controls. To our knowledge, this is the first report suggesting the importance of this factor in delaying AIDS progression in HIV-1 controllers. However, no correlation was observed between *SLPI* expression and viral load or $CD4^+$ T-cell counts.

The mechanisms involved in the antiviral effect of SLPI are not well understood. Initially, it was thought that the effect was mediated by interaction of SLPI with viral envelope proteins, but recently it was shown that SLPI binds to annexin-II, at the macrophage membrane, blocking HIV infection.[32] In fact, annexin-II binds to phosphatidylserine expressed on the HIV-1 envelope, promoting viral binding and entry into target cells; it was demonstrated that annexin-II–deficient cells are less susceptible to HIV infection in vitro.[33]

The results indicate a similar fold increase in *SLPI* expression in response to HIV and LPS in HIV controllers; in

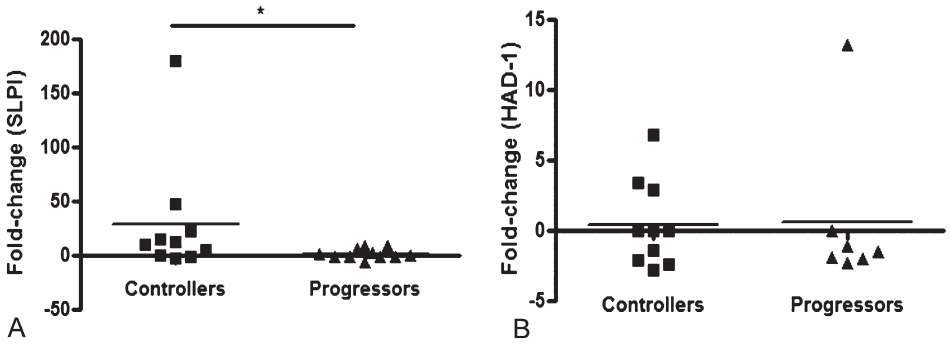

FIGURE 3. *SLPI* and *HAD-1* expression in neutrophils by real-time PCR. RNA from PMNs was extracted and analyzed by real-time PCR for *SLPI* and *HAD-1* expression using the housekeeping gene ubiquitin to normalize the RNA content. The results are presented as median, 25% and 75% percentiles, and range. The statistical comparison between groups was performed using a Mann–Whitney *U* test with a confidence level of 95%. Significant differences are indicated at the top of the figure (*$P < 0.05$).

contrast, a lower fold increase in *SLPI* expression in response to HIV-1, compared with LPS stimulation, was observed in HIV progressors. Previous studies have reported the expression of several pattern recognition receptors by neutrophils, including TLR7[34]; although it was demonstrated that HIV RNA can bind to TLR7[35] and that the signaling through this receptor induces the expression of SLPI,[36] previous reported alterations on TLR expression, specifically a significantly higher expression of TLR4 but not of TLR7, associated with increased HIV RNA load in advanced HIV infection could explain these findings.[37,38]

The production of high amounts of HAD-1 to -3 by mDCs from HIV-1–infected individuals was correlated to the control of viral load, better outcome, and slow disease progression[22]; in our controllers, this association was not observed. This apparent contradiction could be due to genetic/ethnic differences of the individuals included in the survey or to the fact that different cell populations (mDCs vs. PMNs) were analyzed in these 2 studies.

Class I *HLA* alleles have been associated with resistance/susceptibility to several infections, including HIV. However, in the present study we did not find a higher frequency of alleles previously reported to be associated with HIV control; small number of patients comprising each cohort or differences in genetic characteristics of the population included in this study could account for the apparent discrepancy with previous reports.

Immune activation is considered as one of the most important mechanisms mediating apoptosis of lymphocytes. Previous studies have indeed shown that in HIV-infected subjects there is a negative and a positive correlation between immune activation and $CD4^+$ T-cell counts and viral load, respectively,[39–42] and that immune activation is the main pathogenic mechanism associated with AIDS progression. HIV controllers exhibited a lower expression level of the activation markers on $CD4^+$ and $CD8^+$ T cells and mDCs, indicating that the control of immune activation may lead to the control of viral replication and preservation of $CD4^+$ T-cell counts. Similarly, Owen et al[43] reported that LTNPs expressed a lower percentage of $CD8^+$ T cells co-expressing the activation molecules CD38 and HLA-DR.

Although elimination of $CD4^+$ T cells is the hallmark of progressive HIV-1 infection, the frequency and function of other immune subpopulations are altered. iNKT cells are affected at early stages of HIV-1 infection; in progressors, these cells are in low frequency and exhibit a hyperactivated phenotype.[44] In our chronically infected patients, independent of viral load, the frequency of these cells was decreased, suggesting that their loss in PB occurred very early.

Studies evaluating the role of Tregs led to contradictory results, making it difficult to interpret their participation during HIV-1 infection. In this study, HIV-1 controllers exhibited a lower frequency of Tregs compared with progressors. However, HIV-1 controllers do not exhibit a hyperactivated immune status, despite lower levels of Tregs. Low viral loads in these individuals could explain this finding; previous studies have indicated that HIV-1 components, including gp120, induce accumulation of Tregs by expansion of preexisting cells or conversion of naive T cells.[45] In fact, there are reports indicating a positive correlation between viral load and Treg frequency in lymphoid tissue of HIV-1–infected individuals.[46]

In agreement with our results, other studies have demonstrated that accumulation of Tregs or the high expression of FoxP3 is directly correlated to immune activation and AIDS progression.[26,47] Indeed, lower frequency of Tregs should have a beneficial effect during the chronic phase of HIV-1 infection, allowing the effector function of immune cells to take place. However, in contrast to our results, previous reports indicated that a high frequency of Tregs was inversely correlated with immune activation and apoptosis of $CD8^+$ T cells in LTNPs; in addition, the same study reported that decreased Treg frequency was associated with immune cell exhaustion in HIV-1 progressors.[48,49]

Based on our results, we propose a model in which HIV-1 controllers have the ability to produce higher amounts of SLPI, limiting viral replication. Therefore, these individuals maintain a normal frequency of $CD4^+$ T cells and mDCs, preventing extensive damage of gastrointestinal physical barriers, limiting translocation of microbial products from intestinal lumen to systemic circulation. As a result, there is minor cell apoptosis mediated by activation and lower expansion of Tregs, thereby allowing the conservation of innate and adaptive immune components to retard or inhibit AIDS progression.

ACKNOWLEDGMENTS

The authors thank Anne-Lise Haenni for all her constructive comments, and the CIHR IID&GH Program of the University of Manitoba. The H9/HTLVIII cell line was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

REFERENCES

1. Hendriks JC, Medley GF, van Griensven GJ, et al. The treatment-free incubation period of AIDS in a cohort of homosexual men. *AIDS*. 1993; 7:231–239.
2. Lefrere JJ, Morand-Joubert L, Mariotti M, et al. Even individuals considered as long-term nonprogressors show biological signs of progression after 10 years of human immunodeficiency virus infection. *Blood*. 1997; 90:1133–1140.
3. Piacentini L, Fenizia C, Naddeo V, et al. Not just sheer luck! Immune correlates of protection against HIV-1 infection. *Vaccine*. 2008;26: 3002–3007.
4. Walker BD. Elite control of HIV infection: implications for vaccines and treatment. *Top HIV Med*. 2007;15:134–136.
5. Zapata W, Montoya CJ, Rugeles MT. Soluble factors with inhibitory activity against type 1 human immunodeficiency virus [in Spanish]. *Biomedica*. 2006;26:451–466.
6. Modi WS, Lautenberger J, An P, et al. Genetic variation in the CCL18-CCL3-CCL4 chemokine gene cluster influences HIV type 1 transmission and AIDS disease progression. *Am J Hum Genet*. 2006;79:120–128.
7. Velilla PA, Hoyos A, Rojas M, et al. Apoptosis as a mechanism of natural resistance to HIV-1 infection in an exposed but uninfected population. *J Clin Virol*. 2005;32:329–335.
8. Montoya CJ, Velilla PA, Chougnet C, et al. Increased IFN-gamma production by NK and CD3+/CD56+ cells in sexually HIV-1-exposed but uninfected individuals. *Clin Immunol*. 2006;120:138–146.
9. Boulet S, Sharafi S, Simic N, et al. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. *AIDS*. 2008;22: 595–599.

10. Ravet S, Scott-Algara D, Bonnet E, et al. Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. *Blood*. 2007;109:4296–4305.
11. Gillespie GM, Kaul R, Dong T, et al. Cross-reactive cytotoxic T lymphocytes against a HIV-1 p24 epitope in slow progressors with B*57. *AIDS*. 2002;16:961–972.
12. Kelleher AD, Long C, Holmes EC, et al. Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. *J Exp Med*. 2001;193:375–386.
13. Devito C, Broliden K, Kaul R, et al. Mucosal and plasma IgA from HIV-1-exposed uninfected individuals inhibit HIV-1 transcytosis across human epithelial cells. *J Immunol*. 2000;165:5170–5176.
14. Hirbod T, Nilsson J, Andersson S, et al. Upregulation of interferon-alpha and RANTES in the cervix of HIV-1-seronegative women with high-risk behavior. *J Acquir Immune Defic Syndr*. 2006;43:137–143.
15. Pinto LA, Blazevic V, Shearer GM, et al. Alloantigen-induced anti-HIV activity occurs prior to reverse transcription and can be generated by leukocytes from HIV-infected individuals. *Blood*. 2000;95:1875–1876.
16. Quinones-Mateu ME, Lederman MM, Feng Z, et al. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. *AIDS*. 2003;17:F39–F48.
17. Zhang L, Yu W, He T, et al. Contribution of human alpha-defensin 1, 2, and 3 to the anti-HIV-1 activity of CD8 antiviral factor. *Science*. 2002;298:995–1000.
18. Jana NK, Gray LR, Shugars DC. Human immunodeficiency virus type 1 stimulates the expression and production of secretory leukocyte protease inhibitor (SLPI) in oral epithelial cells: a role for SLPI in innate mucosal immunity. *J Virol*. 2005;79:6432–6440.
19. McNeely TB, Dealy M, Dripps DJ, et al. Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. *J Clin Invest*. 1995;96:456–464.
20. McNeely TB, Shugars DC, Rosendahl M, et al. Inhibition of human immunodeficiency virus type 1 infectivity by secretory leukocyte protease inhibitor occurs prior to viral reverse transcription. *Blood*. 1997;90:1141–1149.
21. Mackewicz CE, Yuan J, Tran P, et al. Alpha-defensins can have anti-HIV activity but are not CD8 cell anti-HIV factors. *AIDS*. 2003;17:F23–F32.
22. Rodriguez-Garcia M, Climent N, Oliva H, et al. Increased alpha-defensins 1-3 production by dendritic cells in HIV-infected individuals is associated with slower disease progression. *PLoS One*. 2010;5:e9436.
23. Hazenberg MD, Otto SA, van Benthem BH, et al. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS*. 2003;17:1881–1888.
24. Brenchley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? *Nat Immunol*. 2006;7:235–239.
25. Stevenson M, Stanwick TL, Dempsey MP, et al. HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J*. 1990;9:1551–1560.
26. Cao W, Jamieson BD, Hultin LE, et al. Regulatory T cell expansion and immune activation during untreated HIV type 1 infection are associated with disease progression. *AIDS Res Hum Retroviruses*. 2009;25:183–191.
27. Lee JK, Chae SW, Cho JG, et al. Expression of secretory leukocyte protease inhibitor in middle ear cholesteatoma. *Eur Arch Otorhinolaryngol*. 2006;263:1077–1081.
28. Trabattoni D, Caputo SL, Maffeis G, et al. Human alpha defensin in HIV-exposed but uninfected individuals. *J Acquir Immune Defic Syndr*. 2004;35:455–463.
29. Farquhar C, VanCott TC, Mbori-Ngacha DA, et al. Salivary secretory leukocyte protease inhibitor is associated with reduced transmission of human immunodeficiency virus type 1 through breast milk. *J Infect Dis*. 2002;186:1173–1176.
30. Novak RM, Donoval BA, Graham PJ, et al. Cervicovaginal levels of lactoferrin, secretory leukocyte protease inhibitor, and RANTES and the effects of coexisting vaginoses in human immunodeficiency virus (HIV)-seronegative women with a high risk of heterosexual acquisition of HIV infection. *Clin Vaccine Immunol*. 2007;14:1102–1107.
31. Pillay K, Coutsoudis A, Agadzi-Naqvi AK, et al. Secretory leukocyte protease inhibitor in vaginal fluids and perinatal human immunodeficiency virus type 1 transmission. *J Infect Dis*. 2001;183:653–656.
32. Ma G, Greenwell-Wild T, Lei K, et al. Secretory leukocyte protease inhibitor binds to annexin II, a cofactor for macrophage HIV-1 infection. *J Exp Med*. 2004;200:1337–1346.
33. Ryzhova EV, Vos RM, Albright AV, et al. Annexin 2: a novel human immunodeficiency virus type 1 Gag binding protein involved in replication in monocyte-derived macrophages. *J Virol*. 2006;80:2694–2704.
34. Francois S, El Benna J, Dang PM, et al. Inhibition of neutrophil apoptosis by TLR agonists in whole blood: involvement of the phosphoinositide 3-kinase/Akt and NF-kappaB signaling pathways, leading to increased levels of Mcl-1, A1, and phosphorylated Bad. *J Immunol*. 2005;174:3633–3642.
35. Beignon AS, McKenna K, Skoberne M, et al. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. *J Clin Invest*. 2005;115:3265–3275.
36. Marino R, Thuraisingam T, Camateros P, et al. Secretory leukocyte protease inhibitor plays an important role in the regulation of allergic asthma in mice. *J Immunol*. 2011;186:4433–4442.
37. Lester RT, Yao XD, Ball TB, et al. Toll-like receptor expression and responsiveness are increased in viraemic HIV-1 infection. *AIDS*. 2008;22:685–694.
38. Lester RT, Yao XD, Ball TB, et al. HIV-1 RNA dysregulates the natural TLR response to subclinical endotoxemia in Kenyan female sex-workers. *PLoS One*. 2009;4:e5644.
39. Giorgi JV, Hultin LE, McKeating JA, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis*. 1999;179:859–870.
40. Herbeuval JP, Shearer GM. HIV-1 immunopathogenesis: how good interferon turns bad. *Clin Immunol*. 2007;123:121–128.
41. Liu Z, Cumberland WG, Hultin LE, et al. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997;16:83–92.
42. Moanna A, Dunham R, Paiardini M, et al. CD4+ T-cell depletion in HIV infection: killed by friendly fire? *Curr HIV/AIDS Rep*. 2005;2:16–23.
43. Owen RE, Heitman JW, Hirschkorn DF, et al. HIV+ elite controllers have low HIV-specific T-cell activation yet maintain strong, polyfunctional T-cell responses. *AIDS*. 2010;24:1095–1105.
44. Montoya CJ, Catano JC, Ramirez Z, et al. Invariant NKT cells from HIV-1 or *Mycobacterium tuberculosis*-infected patients express an activated phenotype. *Clin Immunol*. 2008;127:1–6.
45. Ji J, Cloyd MW. HIV-1 binding to CD4 on CD4+CD25+ regulatory T cells enhances their suppressive function and induces them to home to, and accumulate in, peripheral and mucosal lymphoid tissues: an additional mechanism of immunosuppression. *Int Immunol*. 2009;21:283–294.
46. Andersson J, Boasso A, Nilsson J, et al. The prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients. *J Immunol*. 2005;174:3143–3147.
47. Xing S, Fu J, Zhang Z, et al. Increased turnover of FoxP3high regulatory T cells is associated with hyperactivation and disease progression of chronic HIV-1 infection. *J Acquir Immune Defic Syndr*. 2010;54:455–462.
48. Sachdeva M, Fischl MA, Pahwa R, et al. Immune exhaustion occurs concomitantly with immune activation and decrease in regulatory T cells in viremic chronically HIV-1-infected patients. *J Acquir Immune Defic Syndr*. 2010;54:447–454.
49. Jiao Y, Fu J, Xing S, et al. The decrease of regulatory T cells correlates with excessive activation and apoptosis of CD8+ T cells in HIV-1-infected typical progressors, but not in long-term non-progressors. *Immunology*. 2009;128(1 suppl):e366–e375.