Nadir CD4 T Cell Count as Predictor and High CD4 T Cell Intrinsic Apoptosis as Final Mechanism of Poor CD4 T Cell Recovery in Virologically Suppressed HIV-Infected Patients: Clinical Implications

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Background. Although antiretroviral therapy improves immune response, some human immunodeficiency virus–infected patients present unsatisfactory CD4 T cell recovery despite achieving viral suppression, resulting in increased morbidity and mortality.

Methods. Cross-sectional, case-control study to characterize the mechanism and to identify predictive factors of poor immune response. We included 230 patients who were receiving highly active antiretroviral therapy and who had a viral load <50 copies/mL for >2 years; 95 were "discordant" (case patients; CD4 T cell count always <350 cells/μL), and 135 were "concordant" (control subjects). Activation markers, CD4 T cell death (necrosis, intrinsic apoptosis, and extrinsic apoptosis), and caspase-3 were measured. Clinical parameters, particularly antiretroviral combinations, were correlated with immune recovery.

Results. Discordant patients showed higher levels of activation markers, mainly in CD4 T cells ($P < .001$), and higher rates of spontaneous cell death ($P < .001$). Rates of activation and rates of CD4 T cell death (mainly by intrinsic apoptosis) were the best predictive factors for immune recovery, along with nadir CD4 T cell count. Patients who were receiving a protease inhibitor–based regimen were more likely to be discordant and showed higher rates of activation ($P = .011$), higher rates of CD4 T cell death ($P = .033$), and a lower nadir CD4 T cell count ($P < .001$). Multivariate analysis, however, ruled out any effect of protease inhibitors on immune recovery. No differences were observed between the use of tenofovir-emtricitabine (Truvada) and the use of abacavir-lamivudine (Kivexa).

Conclusions. CD4 T cell apoptosis by the intrinsic pathway represents the determinant mechanism of the unsatisfactory immune recovery and should be targeted to manage therapy for discordant patients. The predictive value of low nadir CD4 T cell count for a poor immune recovery led us to consider starting antiretroviral therapy earlier. No differences were observed among antiretrovirals in terms of immune recovery.

Clinicians who care for individuals infected with human immunodeficiency virus (HIV) are concerned about the lack of immune recovery in some virologically suppressed patients. This group, called discordant or immunological nonresponder patients, is defined as patients who are receiving highly active antiretroviral therapy (HAART) and who maintain suppression of HIV replication without an adequate CD4 T cell count recovery. Unlike full responders, discordant patients are at increased risk of clinical progression to AIDS-related and non–AIDS-related illnesses and death [1–7]. Cohort studies reveal a substantial prevalence of immunological nonresponders among patients who are receiving HAART, ranging from 17% to 40%, depending on the study criteria and the population [5, 8, 9]. These data indicate that a high number of treated HIV-infected patients are at risk of clinical progression.

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From a clinical point of view, older age [10, 11], lower nadir CD4 T cell count [1, 10, 12], and hepatitis C virus (HCV) coinfection [13–15] are some of the most relevant predictive factors for a discordant response. Critical questions, such as the choice of the most appropriate antiretroviral therapy in these cases, remain unanswered.

There has been a great deal of effort aimed at understanding the virological and immunological basis for poor immune recovery. In general, low production of new T cells, due to impaired bone marrow or thymus function [1, 16–18], and increased CD4 T cell destruction, revealed by the high sensitivity to cell death ex vivo [17, 18], have been shown to contribute to this failure. Also, the unbalanced T cell homeostasis may be influenced by the existence of latent, undetectable, ongoing viral replication [19], microbial translocation from the gastrointestinal lumen [20], and adverse effects of antiretroviral drugs at the mitochondrial level [21]. Each of these conditions can influence the production, activation, and death of CD4 T cells and thus determine the discordant response.

In this regard, we have recently observed that an increased rate of CD4 T cell death appears to be a determining factor for poor immune recovery in a group of 95 immune-discordant patients, compared with 135 responders (Massanella et al, unpublished data). Such increased destruction of CD4 T cells is closely related to immune hyperactivation, because activated cells are prone to apoptosis ex vivo [22]. However, the ultimate cause of the unfavorable immune response has not been identified. A better knowledge of the definitive pathogenic mechanism and the factors influencing CD4 T cell recovery would help investigators to design adequate strategies focused on improving the immunological response in discordant patients and to determine the optimal therapeutic approach for them.

The present study was designed on the basis of the hypotheses that CD4 T cell apoptosis determines the immune recovery after HAART and that some clinical conditions, such as coinfection or a specific antiretroviral treatment, are triggering factors for discordant response. Accordingly, the aims of this study were to identify the CD4 T cell death pathways that limit recovery of CD4 T cells and to determine their association with clinical parameters and antiretroviral regimens.

MATERIALS AND METHODS

Study design and population. We performed a cross-sectional, case-control study to analyze the role of cell death in discordant immune response. A 12-month period of inclusion (1 November 2007–31 October 2008) was chosen, with the assumption that during this period all discordant patients in our cohort could be identified. We screened 247 consecutive patients who attended our HIV Outpatient Unit, and 230 met the following inclusion criteria: (1) confirmed diagnosis of HIV infection, (2) continual HAART for >2 years that included 2 nucleoside reverse-transcriptase inhibitors (NRTIs) plus 1 ritonavir-boosted protease inhibitor (PI) or 1 nonnucleoside reverse-transcriptase inhibitor (NNRTI) (nevirapine or efavirenz), and (3) sustained undetectable levels of HIV-1 RNA (plasma viral load, <50 copies/mL) for >2 years (minimum number of determinations, 4). The exclusion criteria were chemotherapy, treatment with interferon and/or ribavirin, a history of opportunistic infection during the previous 2 years, and the presence of decompensated liver cirrhosis.

We considered to be discordant (ie, having favorable virological but unfavorable immunological response) those patients with a CD4 T cell count that was always <350 cells/μL. Conversely, concordant patients (favorable virological and immunological response) showed a current CD4 T cell count >400 cells/μL. These criteria ensured that patients with a CD4 T cell count from 350 to 400 cells/μL were excluded from the study, to clearly distinguish the 2 immune scenarios (discordant and concordant response).

The Institutional Review Board of our center approved the study (EO code: EO-07–024). All patients provided their written informed consent.

Study objectives and end points. The main objective of the study was to characterize the cell death pathways that lead to a poor CD4 T cell recovery. Our main end points were the rates of total cell death, necrosis, and apoptosis (intrinsic and extrinsic), compared between discordant and concordant patients. Another objective was the comparison of the levels of CD4 T cell activation and of caspase-3 between discordant and concordant patients. Finally, we investigated the influence of clinical parameters on immune recovery, particularly the effect of different antiretroviral combinations, according to whether they included a PI, an NNRTI, or the most frequently used coformulated NRTIs tenofovir-emtricitabine (Truvada) or abacavir-lamivudine (Kivexa).

Assessments. Information on patient characteristics and HIV-related data was collected from medical records. The status of infection with hepatitis B virus (HBV) or with HCV was also retrieved from this patient history database.

A single blood sample was drawn from each participant. Blood was immediately stained and processed. Plasma was obtained by centrifugation of blood at 1200 *g* for 10 minutes and was stored at −80°C. Peripheral blood mononuclear cells (PBMCs) were obtained from cell concentrates layered on Ficoll–Hypaque density gradients (Atom Reactiva) and were used immediately for ex vivo cell death assays or were frozen for caspase-3 determinations.

Cell death was evaluated by culturing aliquots of 200,000 PBMCs in 96-well plates in 100 μL of Roswell Park Memorial Institute medium (containing 10% fetal calf serum) for 0, 1, and 4 days in the absence or presence of the pancaspase inhibitor Z-VAD-fmk (R & D Systems). Cells were analyzed in

an LSRII flow cytometer (Becton Dickinson) after incubation with 40 nM of the potentiometric mitochondrial probe $DiOC_6$ (Invitrogen), 5 μg/mL propidium iodide (Sigma), and CD3-APC-Cy7, CD4-APC, and CD8-PE-Cy7 antibodies. Total cell death was calculated as the percentage of cells that showed low $DiOC_6$ staining in control cultures [23].

On day 1, we performed additional analyses of necrotic cell death (caspase-independent), which was defined as the percentage of propidium iodide–stained cells in cultures that contained Z-VAD-fmk. Apoptotic cell death was defined as caspase-dependent death and was calculated by subtracting necrosis from total cell death. Intrinsic apoptosis was defined as the percentage of cells that showed low $DiOC_6$ staining and that remained negative for propidium iodide in the presence of Z-VAD-fmk. Extrinsic apoptosis was calculated as the difference between total and intrinsic apoptosis. Caspase-3 activity was determined using 15 μg of total protein from PBMC lysates with a fluorometric assay (Ac-DEVD-AMC, CASPASE-3; Becton Dickinson).

CD4 and CD8 T cell immunophenotypes were assessed by staining fresh blood samples with the following antibody combination: CD95-FITC, PD-1-PE, HLA-DR-PerCP, CD3-APC-Cy7, CD4-APC, and CD8-PE-Cy7, which was designed to evaluate activation. A control staining and a control combination that contained CD3-APC-Cy7, CD4-APC, and CD8-PE-Cy7 antibodies were performed for all samples. Cells were acquired in the LSRII flow cytometer and were analyzed with FlowJo software (Tree Star).

Soluble CD14 levels, which are a surrogate marker for bacterial translocation [24], were quantified in all plasma samples by means of commercially available enzyme-linked immunosorbent assays (Diaclone). Plasma samples were diluted (1:50) and were tested in duplicate.

Statistical analysis. Continuous variables were expressed as the median (interquartile range [IQR]) and were compared using nonparametric tests (Mann-Whitney for nonpaired data and Wilcoxon for paired data), because the parameters were not normally distributed. Discrete variables were described as number of patients (percentage), and the χ^2 or Fisher exact test was used, as appropriate. The Pearson correlation coefficient was calculated to assess the association between the apoptosis parameters and the clinical variables.

Univariate and multivariate logistic regressions were fitted to predict the probability of discordance by considering the following as explanatory variables: age, sex, route of transmission, time with HIV infection, time receiving antiretroviral therapy, antiretroviral used at baseline, time with suppressed viral load, nadir CD4 T cell count, baseline CD4 T cell count, coinfection with HBV or HCV, and hepatitis C viral load. The models were also fitted after adjustment for use of PI and for HCV coinfection.

Statistical analyses were performed using SPSS, version 15.0 (SPSS), with univariate 2-tailed significance levels of .05. Graphs were plotted with GraphPad Prism, version 5 (GraphPad).

RESULTS

Patient characteristics. A total of 230 patients were included in the study: 95 were defined as discordant and 135 as concordant. Most demographic and clinical parameters were well balanced among both groups (Table 1). However, minimal but significant differences were observed in the time with viral load <50 copies/mL and in CD8 T cell absolute counts. As expected, significantly lower CD4 T cell counts (absolute and percentage), significantly lower nadir CD4 T cell counts, and a significantly higher number of patients with nadir CD4 T cell counts <200 cells/μL were observed in the discordant group ($P < .001$ for all). Patients with HCV coinfection and patients who were receiving PI-containing regimens were more likely to be found in the discordant group (Table 1).

Analysis of activation and destruction and characterization of cell death pathways. Cell death was measured in CD4 and CD8 T cells immediately after cell purification (day 0) and after 1 or 4 days of ex vivo culture. In particular, levels of an activation marker ($CD4^+HLA\text{-}DR^+CD95^+$) and rates of total death in CD4 T cells were significantly higher in discordant patients ($P < .001$) (Table 1 and Figure 1A). Conversely, although CD8 T cells showed increased levels of activation markers ($CD8^+HLA\text{-}DR^+CD95^+$) (Table 1), no significant differences in rates of ex vivo cell death were observed in this subset (Figure 1A).

With regard to the mechanisms of cell death, the discordant group showed higher rates of necrosis and of total apoptosis (intrinsic and extrinsic) in CD4 T cells at day 1, compared with the corresponding rates for concordant patients (Figure 1B). In contrast, CD8 T cells from discordant patients showed higher rates of necrosis and intrinsic apoptosis but lower rates of extrinsic apoptosis (Figure 1C). Levels of caspase-3 were significantly higher in discordant patients (10,409 absorbance units/mg protein [IQR, 7018–16,203 absorbance units/mg protein]) than in concordant patients (9035 absorbance units/mg protein [IQR, 5080–13,358 absorbance units/mg protein]) ($P = .050$).

Rates of CD4 T cell intrinsic apoptosis showed a stronger correlation than did rates of necrosis or extrinsic apoptosis with the expression of different markers of activation, such as the frequency of $CD38^+CD45RA^-$ CD4 T cells (r, 0.617; $P < .001$) and $HLA\text{-}DR^+CD95^+$ CD4 T cells (r, 0.523; $P < .001$). This finding suggests that activated cells die mainly by intrinsic apoptosis.

Clinical parameters that influenced cell death and discordant response. On inclusion, 55% of participants were receiving a stable PI-based regimen, mainly atazanavir (44.3%) and lopinavir-ritonavir (44.3%), and 45% were receiving an NNRTI-based regimen. Patients who were receiving a PI-con-

Table 1. HIV-Related Characteristics and Activation Markers for Concordant and Discordant Patients

Variable	Concordant patients (n = 135)	Discordant patients (n = 95)	P
Age, years	43 (39–50)	46 (42–50)	.061
Male sex, no. (%) of patients	100 (74)	78 (82)	.200
Time since HIV diagnosis, months	155 (108–197)	170 (88–242)	.310
Time receiving ART, months	131 (95–161)	114.5 (55–169)	.165
Time with VL <50 copies/mL, months	49 (31–73)	40 (27–60)	.030
Nadir CD4 T cell count, cells/μL	234 (132–319)	71 (28–135)	<.001
Nadir CD4 T cell count <200 cells/μL, no. (%) of patients	61 (45)	85 (90)	<.001
Absolute CD4 T cell count, cells/μL	632 (480–796)	249 (200–319)	<.001
CD4 T cell count, %	30 (26–36)	18 (14–21)	<.001
Time to achieve current CD4 T cell count, months[a]	131 (95–157)	113 (55–169)	.200
Absolute CD8 T cell count, cells/μL	805 (648–1112)	724 (510–986)	<.001
CD8 T cell count, %	51 (45–58)	41 (35–48)	.023
Current ART, no. (%) of patients			
PI	50 (37)	53 (55)	.007
NNRTI	84 (63)	43 (45)	.015
TDF-FTC (Truvada)	68 (50)	47 (49)	.187
ABV-3TC (Kivexa)	28 (21)	23 (24)	.165
HCV coinfection, no. (%) of patients	43 (32)	44 (46)	.028
HBV coinfection, no. (%) of patients	6 (4)	4 (4)	>.99
$CD8^+HLA\text{-}DR^+CD95^+$, % of CD8 T cells	8.2 (4.3–11.6)	12.4 (6.2–19.6)	<.001
$CD4^+HLA\text{-}DR^+CD95^+$, % of CD4 T cells	4.7 (3.2–6.9)	10.1 (6.7–19.2)	<.001

NOTE. Data are median (interquartile range), unless otherwise indicated. All participants had a human immunodeficiency viral load <50 copies/mL for >2 years. Discordant patients were defined by a CD4 T cell count always <350 cells/μL. Concordant patients were defined by a CD4 T cell count always >400 cells/μL. ABV, abacavir; ART, antiretroviral treatment; FTC, emtricitabine; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NNRTI, nonnucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir; VL, viral load; 3TC, lamivudine.

[a] Time from starting antiretroviral to current CD4 T cell count.

taining regimen were mostly in the discordant group (Table 1) and showed a lower nadir CD4 T cell count, a higher proportion of patients with nadir CD4 T cell count <200 cells/μL, a longer period with HIV infection, a shorter time with viral suppression, and more common HCV coinfection (Table 2). Significant differences were also seen in levels of activation markers ($CD8^+CD38^+CD45RA^-$, $P = .011$), rates of CD4 T cell death ($P = .033$), levels of soluble CD14 ($P = .002$), and rates of total ($P = .041$), intrinsic ($P = .038$), and extrinsic ($P = .033$) apoptosis (Table 2). CD8 T cells showed similar characteristics in both groups (data not shown).

No differences were observed between patients who were receiving tenofovir-emtricitabine and patients who were receiving abacavir-lamivudine with regard to immune recovery, T cell production, activation, or destruction (data not shown).

Compared with patients without HCV coinfection, patients with HCV coinfection were more likely to be discordant (Table 1) and presented a lower nadir CD4 T cell count ($P = .021$), longer time with HIV infection ($P = .017$), and higher levels of immune markers ($HLA\text{-}DR^+CD95^+$ CD4 T-cells, $P = .003$; and $CD38^+CD45RA^-$ CD8 T-cells, $P = .017$) and of soluble CD14 ($P < .001$). CD4 T cell destruction and production were similar between patients with and patients without HCV coinfection.

Univariate and multivariate analyses. In the univariate analysis (Figure 2A), there were statistically significant associations between poor immune recovery and low nadir CD4 T cell count, HCV coinfection, and PI use. The rate of death in the CD8 T cell subset was unrelated to or poorly predictive of the discordant phenotype. However, the same parameters measured in CD4 T cells were statistically significant. Of note, the rate of CD4 T cell death at day 0 and the rates of necrosis and intrinsic apoptosis at day 1 were the best predictors of discordance. No association was seen with age, sex, time with HIV infection, time with viral suppression, or type of NNRTI used.

In the multivariate analysis (Figure 2B), discordance was more likely in patients who presented a low nadir CD4 T cell count (odds ratio [OR], 1.739; 95% confidence interval [CI], 1.351–2.129; $P < .001$), high rates of necrosis in CD4 T cells (OR, 1.515; 95% CI, 1.053–2.180; $P = .025$), and high rates of CD4 T cell intrinsic apoptosis at day 1 (OR, 2.266; 95% CI, 1.455–3.531; $P < .001$).

These increased risks changed slightly after adjustment for PI use: low CD4 nadir T cell counts (OR, 1.653; 95% CI, 1.351–

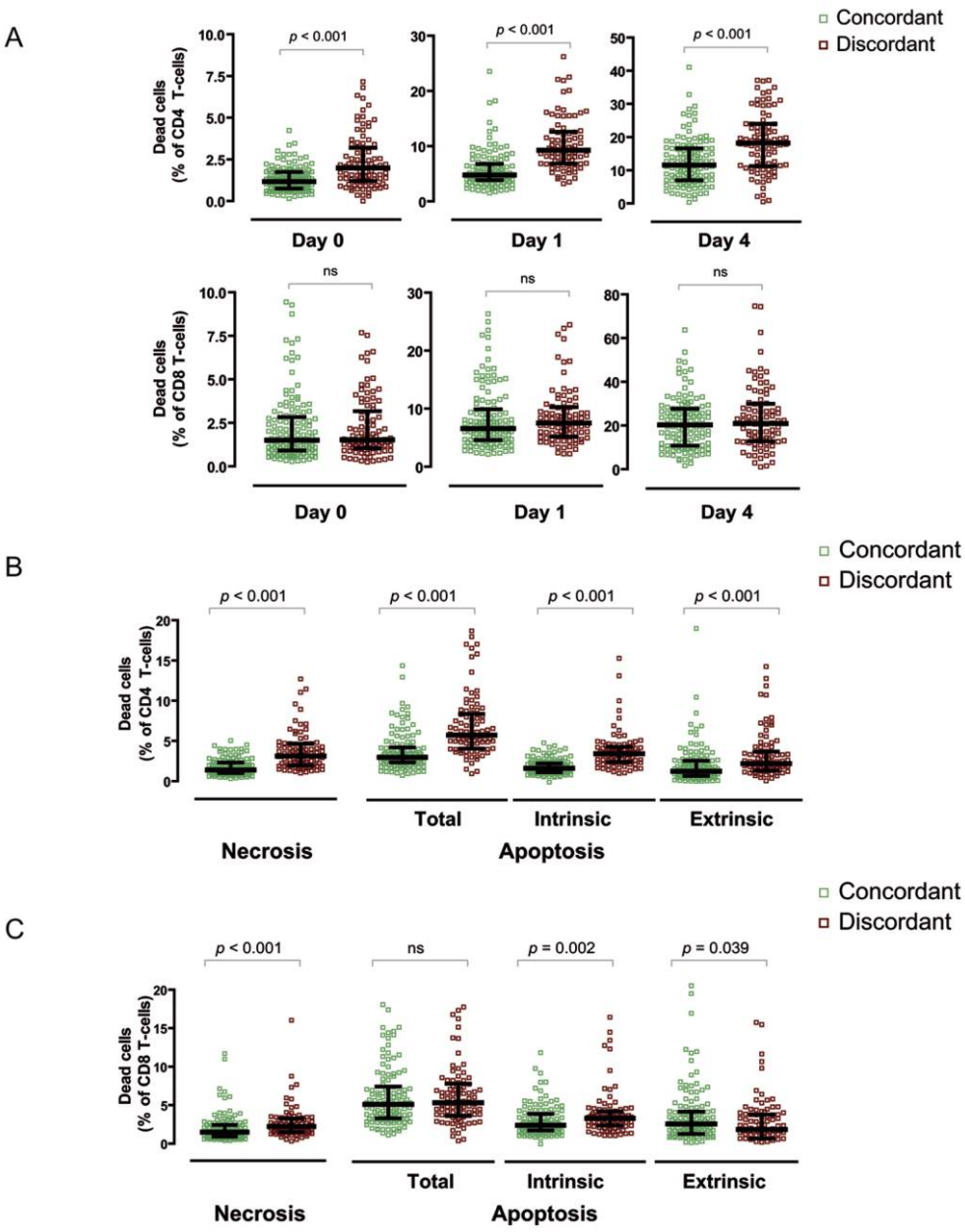

Figure 1. Analysis of cell death mechanisms. Total cell death in CD4 T cells and CD8 T cells was evaluated after 0, 1, and 4 days ex vivo cultures of peripheral blood mononuclear cells (PBMCs) *(panel A)*. Different mechanisms of cell death (necrosis, total apoptosis, intrinsic apoptosis, and extrinsic apoptosis) in CD4 T cells *(panel B)* and in CD8 T cells *(panel C)* were analyzed after 1 day ex vivo cultures of PBMCs. Individual measures of concordant patients *(green symbols)* or discordant patients *(red symbols)* with the median values *(black lines)* and interquartile ranges *(black bars)* are shown. *P* values were calculated with the Mann-Whitney test.

2.129; $P < .001$), high rates of necrosis of CD4 T cells at day 1 (OR, 1.523; 95% CI, 1.057–2.194; $P = .024$), and high rates of CD4 T cell intrinsic apoptosis (OR, 2.277; 95% CI, 1.458–3.555; $P < .001$). Because the criterion for the identification of discordant patients was based on absolute CD4 T cell counts, we investigated the role of intrinsic apoptosis in CD4 T cell recovery after stratification of all patients ($n = 230$) according to their increase in CD4 T cells (difference between their current and nadir CD4 T cell counts). Higher increases in CD4 T cells were associated with lower intrinsic apoptosis ($P < .001$) (Figure 2C).

DISCUSSION

A decreased thymic production and an increased activation and death of CD4 T cells are some of the proposed mechanisms to

Table 2. Characteristics of HIV-Infected Patients according to Regimen Based on Protease Inhibitor (PI) or Nonnucleoside Reverse-Transcriptase Inhibitor (NNRTI)

Characteristic	PI-based regimen (n = 103)	NNRTI-based regimen (n = 127)	P
Age, years	44 (41–48)	45 (40–51)	.613
Male sex, no. (%) of patients	78 (76)	100 (79)	.636
Time since HIV diagnosis, months	175 (123–230)	143 (27–206)	.017
Time with VL <50 copies/mL, months	38 (23–63)	51 (34–74)	.001
Nadir CD4 T cell count, cells/μL	92 (39–185)	189 (91–304)	<.001
Nadir CD4 T cell count <200 cells/μL, no. (%) of patients	80 (78)	66 (52)	<.001
HCV coinfection, no. (%) of patients	55 (53)	32 (25)	<.001
Discordant, no. (%) of patients	58 (56)	47 (37)	.007
$CD4^+CD45RA^+CD31^+$, % of CD4 T cells	17.4 (8.5–27.5)	15.6 (7.4–22.8)	.148
$CD4^+HLA\text{-}DR^+CD95^+$, % of CD4 T cells	7.0 (3.9–12.6)	6.1 (4.2–8.9)	.225
$CD8^+CD38^+CD45RA^-$, % of CD8 T cells	28.0 (18.7–39.3)	22.9 (16.8–31.4)	.011
Soluble CD14, μg/mL	8.9 (7.7–10.5)	8.0 (6.9–9.3)	.002
Total cell death, % of CD4 T cells	6.95 (4.74–10.33)	6.12 (4.14–8.93)	.033
Necrosis, % of CD4 T cells	2.24 (1.34–3.50)	1.89 (1.21–3.28)	.127
Apoptosis, % of CD4 T cells	3.79 (2.56–5.64)	3.32 (2.09–4.89)	.041
Intrinsic apoptosis, % of CD4 T cells	2.45 (1.72–3.76)	2.08 (1.44–3.36)	.038
Extrinsic apoptosis, % of CD4 T cells	2.08 (1.17–3.81)	1.59 (0.85–2.78)	.033

NOTE. Data are median (interquartile range), unless otherwise indicated. HCV, hepatitis C virus; HIV, human immunodeficiency virus; VL, viral load.

explain the failure to repopulate CD4 T cells in some HIV-infected patients despite an adequate virologic response to HAART [5]. Our findings support these theories and point to intrinsic apoptosis of CD4 T cell death as the predominant mechanism of cell destruction and the determining factor of discordant immune response. Clinical implications that emerge from our findings may help to answer 2 major questions about HIV infection: when to start antiretroviral therapy and what specific therapy to choose.

An increased rate of T lymphocyte cell death is one of the many adverse consequences of HIV infection and a major factor contributing to immune deterioration [25]. Cell death can be observed in multiple cell types, particularly in CD4 T cells, in which apoptosis plays a determinant role [22, 26, 27]. Antiretroviral treatment induces a decrease in rates of apoptosis as a result of a reduction in viral replication, which leads to decreased levels of viral proteins that are implicated in apoptosis and immune activation. This reduction contributes to the immune recovery that is associated with HAART. However, ~30% of patients present a discordant response to treatment, maintaining a low CD4 T cell count despite viral suppression [5, 8, 9]. This discordant immune response, accompanied by the worse outcome and the faster clinical evolution observed in these patients, has been the cause of a wide investigation [1–7].

Although the origins of the activated phenotype and of the increase in rates of CD4 T cell death remain unclear in discordant patients, there is enough evidence to confirm the strong link between activation and cell death, characterized by the high tendency of activated cells to undergo apoptosis [22]. Higher caspase-3 levels were observed in our discordant group, thus supporting higher rates of total apoptosis in these patients. In addition, our analysis shows that intrinsic apoptosis is the predominant pathway of activated human CD4 T cell destruction, as reported for animal models [28], although the extrinsic apoptotic pathway and necrosis are also involved. Interestingly, higher rates of necrosis and intrinsic apoptosis of CD8 T cells were also observed in discordant patients, which shows higher activation rates in this subset. This fact reinforces the notion that increased rates of activation are linked to higher sensitivity to intrinsic pathways of apoptosis.

In clinical terms, the knowledge of the pathogenesis of CD4 T cell destruction is a basic step in the design of successful strategies to improve immune reconstitution in immunological nonresponders. The predominant implication of intrinsic apoptosis in poor immune recovery makes sense, because antiretroviral drugs may inhibit or activate apoptosis, thus influencing treatment efficacy. PIs, in particular, modulate apoptosis by preventing the opening of the mitochondrial membrane in animal models [29, 30] and have been associated with lower rates of CD4 T cell apoptosis in HIV-infected patients [31–34]. However, these data remain controversial [35, 36]. Surprisingly, in our study, patients who received a PI-based regimen showed higher apoptotic activity. This apparent paradox could be explained by the fact that these patients had a significantly worse clinical condition (including predictors of discordant immune

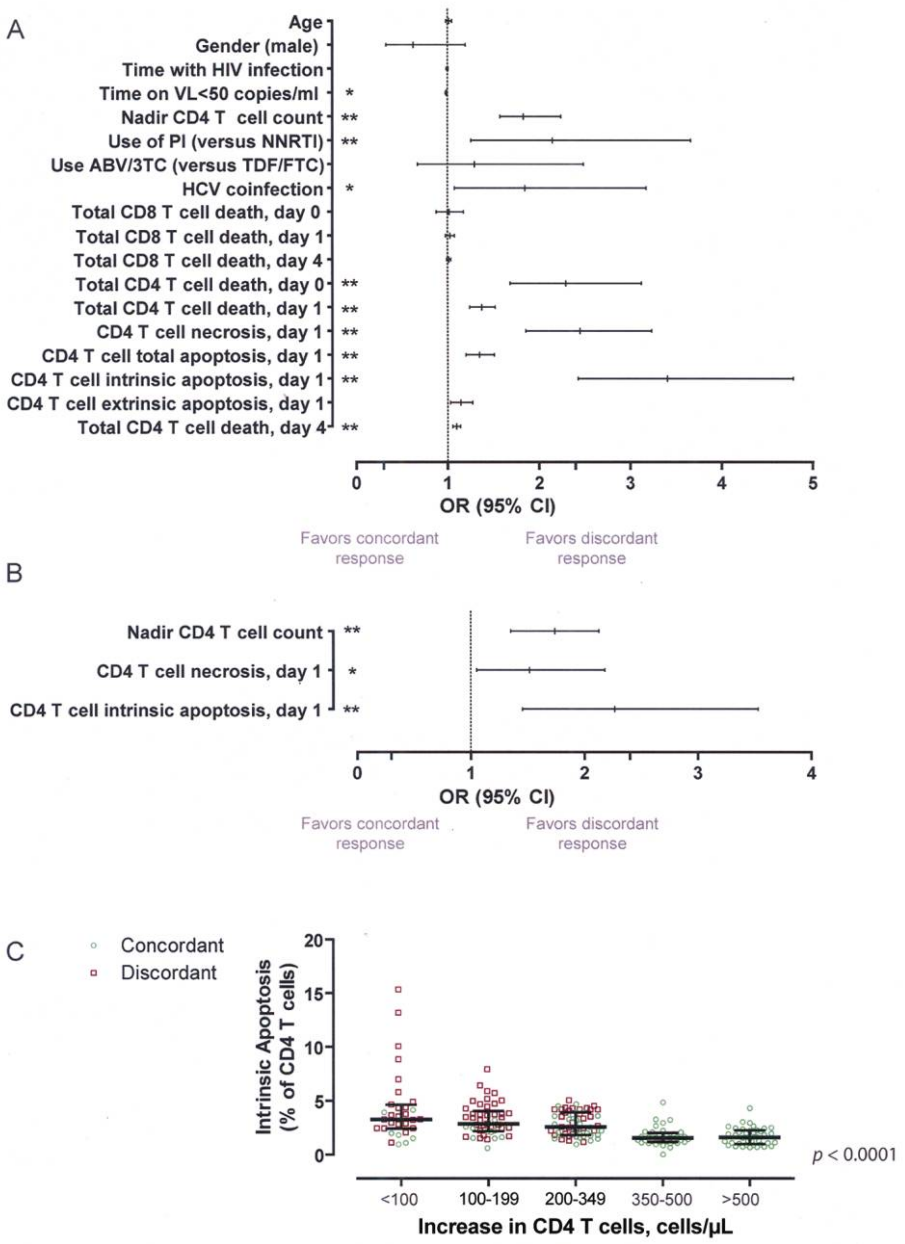

Figure 2. Predictive factors for unsatisfactory immune recovery. The ability of the indicated parameters to predict the probability of discordance was assessed in *A,* univariate analysis and *B,* multivariate analysis, which included most of parameters from the univariate analysis. *Points* denote odds ratios (ORs), and *lines* indicate 95% confidence intervals (CIs). ORs for nadir CD4 T cell counts were calculated for decreases of 50 cells/μL. Asterisks denote statistical significance: * $P < .05$, ** $P < .005$. ABV, abacavir; FTC, emtricitabine; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NNRTI, nonnucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir; VL, viral load; 3TC, lamivudine. *C,* Patients were stratified according to their recovery of CD4 T cells, measured as the difference between their current CD4 T cell count and their nadir value. Five groups were defined by the increase in CD4 T cell count of <100 cells/μL ($n = 31$), 100–199 cells/μL ($n = 47$), 200–349 cells/μL ($n = 61$), 350–500 cells/μL ($n = 44$), and >500 cells/μL ($n = 42$). Intrinsic apoptosis of CD4 T cells after 1 day of ex vivo culture was analyzed. Dots indicate individual data; for illustrative purposes, discordant patients are shown as *red squares* and concordant patients as *green circles*. Median and interquartile range are shown for each group; the P value, calculated with the Kruskal-Wallis test, applies to differences among groups.

response, such as lower nadir CD4 T cell count, longer time of HIV infection, and higher rate of HCV coinfection) that probably led physicians to prefer a PI-containing regimen. Multivariate analysis, however, ruled out the use of a PI as a risk factor or as a protective factor for a discordant response. Similarly, no association was observed between use of abacavir-lamivudine or tenofovir-emtricitabine and immune response. Given these results, it seems reasonable that there are no apparent differences

between the most common antiretroviral combinations in terms of rates of apoptosis and immune response.

Many clinical factors have been related to immune recovery [5]. Our analysis demonstrates that low nadir CD4 T cell count remains the strongest factor associated with a discordant immune response and was firmly associated with immune activation and rates of intrinsic apoptosis. However, immune response was not significantly related to other parameters (eg, age, time with viral replication, or presence of HCV infection). Particularly, HCV coinfection, whose effect on immune recovery is controversial [5, 15], was not relevant in our multivariate analysis. Therefore, our data suggest that an early initiation of antiretroviral therapy in HIV-infected patients is a feasible intervention to prevent the immune deterioration that leads to unsatisfactory immune recovery.

In summary, our experimental data establish that CD4 T cell hyperactivation and the intrinsic pathway of cell death represent the determinant and final mechanisms of the unsatisfactory immune recovery in discordant patients and should be targeted to better manage treatment of these patients with more appropriate strategies. We believe that essential clinical implications of HIV infection could emerge from our data to help address such relevant decisions as when to start antiretroviral therapy and what specific therapy to choose. Because nadir CD4 T cell count was the main predictive factor of immune response and because current antiretroviral combinations had similar effects on immune recovery, we believe that HAART should be initiated earlier and that any common HAART combination can be used. Although this was a large cohort, our data arise from a cross-sectional study. Therefore, these results should be supported by further studies designed for this purpose.

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