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Differential Effects of Viremia and Microbial Translocation on Immune Activation in HIV-Infected Patients Throughout Ritonavir-Boosted Darunavir Monotherapy

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Abstract: The purpose of this article is to evaluate the evolution of microbial translocation (MT) and its role in $CD4^+$ and $CD8^+$ T cells immune activation (IA) in HIV-1-infected patients on ritonavir-boosted darunavir monotherapy (mtDRV/rtv).

Prospective study of consecutive HIV-1-infected patients switched to mtDRV/rtv as a simplification regimen. Subjects were classified according to the virological behavior during a 24-month follow-up as continuous undetectable viral load, blips, intermittent viremia, and virological failure (VF). MT was evaluated by plasma LPS and 16S genomic rDNA (16S rDNA) levels, whereas IA was assessed by the coexpression of HLA-DR and CD38 in $CD4^+$ and $CD8^+$ T cells, and plasma sCD14 levels.

Seventy-one patients were included in this substudy of the MonDar cohort (ClinicalTrials.gov: NCT01505722). At baseline, $CD4^+$ ($\rho = -0.352$, $P = 0.01$) and $CD8^+$ T-cell activation ($\rho = -0.468$, $P < 0.001$) were correlated with time with viral suppression, but not with MT markers. A significant decrease in plasma LPS levels was found only in patients without VF (baseline, 77.8 vs month 24, 60.4 pg/mL; $P < 0.001$]. Both plasma 16S rDNA and sCD14 levels were unchanged irrespective of the viral behavior. The only variable independently associated with a decrease in $CD4^+$ and $CD8^+$ T cells activation was an undetectable HIV-1 viremia ($\beta = 4.78$, $P < 0.001$ and $\beta = 2.93$, $P = 0.005$, respectively).

MT does not have a pivotal role in T-cell activation, at least in patients with long-term viral suppression. The viremic episodes and VF are the main factors related to $CD4^+$ and $CD8^+$ T-cells IA, even during mtDRV/rtv.

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Abbreviations: 16S rDNA = 16S genomic rDNA, CUV = continuous undetectable viremia, IA = immune activation, IV = intermittent viremia, LPS = lipopolysaccharide, MT = microbial translocation, mtDRV/rtv = ritonavir-boosted darunavir monotherapy, mtPI/rtv = ritonavir-boosted protease inhibitor-based monotherapy, VF = virological failure.

INTRODUCTION

Several studies have highlighted the importance of microbial translocation (MT) as a pivotal mechanism for persistent immune activation (IA) during HIV-1 infection.[1–4] This anomalous MT arises as a direct consequence of severe $CD4^+$ T-cell depletion in the gastrointestinal tract and damage to the integrity of the intestinal mucosa.[5–7] MT can be measured in plasma by direct quantification of bacterial products, including lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, as well as conserved sequences of 16S genomic rDNA (16S rDNA) common to most bacteria or other microbe-specific compounds. Both of them were positively correlated with $CD8^+$ T-cell activation in untreated HIV infection and several longitudinal studies have shown important decreases in plasma LPS, 16S rDNA, and sCD14 levels after the initiation of ART, though without reaching the values of healthy individuals.[1,8–14]

In the past few years, ART simplification strategies, such as ritonavir-boosted protease inhibitor-based monotherapy (mtPI/rtv), have been explored in patients with long-standing virological suppression. Data from several clinical trials of lopinavir/ritonavir and ritonavir-boosted darunavir monotherapy (mtDRV/rtv) have shown that mtPI/rtv has a similar or slightly less efficacy than triple therapy to maintain viral load suppression.[15–19] Even so, there is significant controversy regarding the use of this therapeutic strategy,[20–25] and information about the impact of this therapeutic option on issues other than virological efficacy and safety is still scarce. Herein, we have assessed the evolution of MT and its role in $CD4^+$ and $CD8^+$ T cells IA profile in HIV-1-infected patients on mtDRV/rtv over a 24-month period.

MATERIAL AND METHODS

As previously described in detail,[26,27] the MonDar cohort (Clinical-Trials.gov identifier: NCT01606722) prospectively enrolled 150 HIV-1-infected patients with virological suppression for $\geq$6 months who were switched to mtDRV/rtv (800/100 mg once daily) as a simplification strategy from January 2010 to April 2011. The study was conducted after obtaining informed consent according to the principles of the Declaration of Helsinki. The study was approved by the Ethics Committee in

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Patients, Follow-Up, and Samples

Clinical assessments and sampling were performed just before switching to mtDRV/rtv and quarterly thereafter, including biochemical and hematological profiles, $CD4^+$ T cells counts, and plasma HIV-1 viremia (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0; Roche, Branchburg, NJ). In this substudy, only patients with available samples just before and at month 6, 12, 18, and 24 after switching to mtDRV/rtv were included. Patients who presented virological failure (VF) on mtDRV/rtv were also followed up for 24 months after reachieving virological control by triple therapy reintroduction or by encouraging adherence.

Patients were classified according to their virological behavior into 4 groups: continuous undetectable viremia (CUV) (<20 copies/mL); blips or transitory episodes of HIV-RNA >20 copies/mL, preceded and followed by undetectable viremia without changes in ART; VF, defined as 2 consecutive HIV-RNA >200 copies/mL; and intermittent viremia (IV) defined as episodes of detectable HIV-RNA during the follow-up yet without meeting the blip or VF criteria.

Laboratory Procedures

All samples were tested in duplicate. Plasma LPS and sCD14 were measured by the QCL-1000 Limulus Amebocyte Lysate kit (Lonza, Basel, Switzerland) and the Human sCD14 Quantikine ELISA kit (R&D Systems, Abingdon, Oxfordshire, UK), respectively, following manufacturer's instructions. Plasma 16S rDNA was measured by quantitative polymerase chain reaction by using degenerate forward and reverse primers (8F: 5′-AGAGTTTGATYMTGGCTCAG-3′; 361R: 5′-CGYCCATTGBGBAADATTCC-3′) and a TaqMan probe (338P: 5′-FAM-TACGGGAGGCAGCAGT-BHQ1-3′).[10] The IA profile was measured as the coexpression of HLA-DR and CD38 in both $CD4^+$ and $CD8^+$ T cells as previously described.[28]

Statistical Analysis

Results are expressed as median and interquartile range for continuous variables and as number of cases and percentages for categorical variables. The Kruskal–Wallis and analysis of variance tests were performed to compare continuous variables, whereas the χ^2 and Fisher exact tests were run to compare categorical variables. The relationships between continuous variables were assessed by Spearman rank correlation coefficients (ρ test). The variables that were statistically significant in the univariate analysis were included in a multiple linear regression model. The differences were considered statistically significant for P values <0.05. The statistical analyses were performed using SPSS software (v. 19.0, Chicago, IL).

RESULTS

A total of 71 patients were included in this study and classified as CUV: 19 (26.8%); blips: 15 (21.1%); IV: 21 (29.6%); and VF: 16 (22.5%). Patients' baseline characteristics are shown in Table 1. At baseline, a weak correlation was found between time with viral suppression and plasma levels of LPS ($\rho = -0.311$, $P = 0.03$), but not with 16S rDNA ($\rho = 0.051$, $P = 0.67$) or sCD14 ($\rho = -0.075$, $P = 0.53$). Moreover, time with viral suppression was inversely correlated with the percentages of HLA-DR^+CD38^+ $CD4^+$ ($\rho = -0.352$, $P = 0.01$) and HLA-DR^+CD38^+ $CD8^+$ T cells ($\rho = -0.468$, $P = 0.001$). The IA of $CD4^+$ and $CD8^+$ T cells were correlated with the sCD14 levels ($\rho = 0.277$, $P = 0.05$ and $\rho = 0.412$, $P = 0.003$), but not with LPS or 16S rDNA levels. Other epidemiological factors, such as age or hepatitis C virus coinfection, did not correlate with any of the MT or IA markers.

MT Evolution Over 24 Months on mtDRV/rtv

A significant decrease in plasma LPS levels was found in patients without VF (CUV, blips, and IV groups) (month 0, 77.8 [51.6–108.96] vs month 24, 60.4 pg/mL [51.8–74.4], $P = 0.006$; Figure 1A). By contrast, in those patients who experienced VF, the LPS levels remained unchanged in comparison with baseline values both at VF time and at month 24,

TABLE 1. Patients' Baseline Characteristics

	CUV (n = 19)	Blips (n = 15)	IV (n = 21)	VF (n = 16)	*P*
Age, y; M (IQR)	47 (42–50)	47 (38–51)	44 (39–51)	46 (42–49)	0.87
Sex (male), n (%)	14 (73.7)	11 (73.3)	19 (90.5)	10 (62.5)	0.25
Chronic hepatitis C, n (%)	4 (21.1)	4 (26.7)	4 (19.0)	4 (25.0)	0.90
Time on HAART, mo; M (IQR)	117 (86–160)	59 (42–136)	100 (66–130)	83 (47–122)	0.30
Time with viral suppression, mo; M (IQR)	68 (40–97)	39 (30–97)	51 (34–84)	42 (16–68)	0.48
$CD4^+$ T cell counts, cell/μL; M (IQR)	545 (388–713)	592 (502–908)	560 (327–897)	598 (435–784)	0.50
Nadir $CD4^+$ T cell counts, cell/μL; M (IQR)	163 (32–224)	144 (68–225)	177 (94–264)	125 (37–222)	0.37
Zenith VL, $\log_{10}$ copies/mL; M (IQR)	5.00 (4.63–5.78)	6.05 (5.10–6.21)	5.17 (4.97–5.36)	5.2 (5.0–5.5)	0.06
Blips in the previous 12 mo, n (%)	2 (10.5)	5 (33.3)	8 (38.1)	5 (31.3)	0.15
HLA-DR^+CD38^+ $CD4^+$ T cells, %; M (IQR)	4.6 (3.9–6.8)	5.1 (3.5–7.0)	4.8 (2.7–6.7)	6.1 (4.5–7.2)	0.38
HLA-DR^+CD38^+ $CD8^+$ T cells, %; M (IQR)	5.0 (4.3–8.6)	6.2 (4.5–8.5)	5.8 (3.9–7.5)	7.4 (6.3–10.6)	0.09
sCD14, pg/mL; M (IQR)	10.9 (8.6–13.2)	11.2 (10.1–12.3)	10.9 (8.8–13.2)	10.1 (9.4–13.9)	0.89
LPS, pg/mL; M (IQR)	79.3 (62.1–145.8)	63.8 (42.9–172.3)	78.66 (54.0–102.0)	76.1 (53.3–109.8)	0.92
16S rDNA, $\log_{10}$ copies/mL; M (IQR)	5.4 (4.0–6.0)	5.4 (5.6–5.8)	5.3 (4.6–5.7)	5.3 (5.0–5.4)	0.68

CUV = continuously undetectable viremia, HAART = highly active antiretroviral therapy, IQR = interquartile range, IV = intermittent viremia, LPS = lipopolysaccharide, M = median, VF = virological failure, VL = viral load.

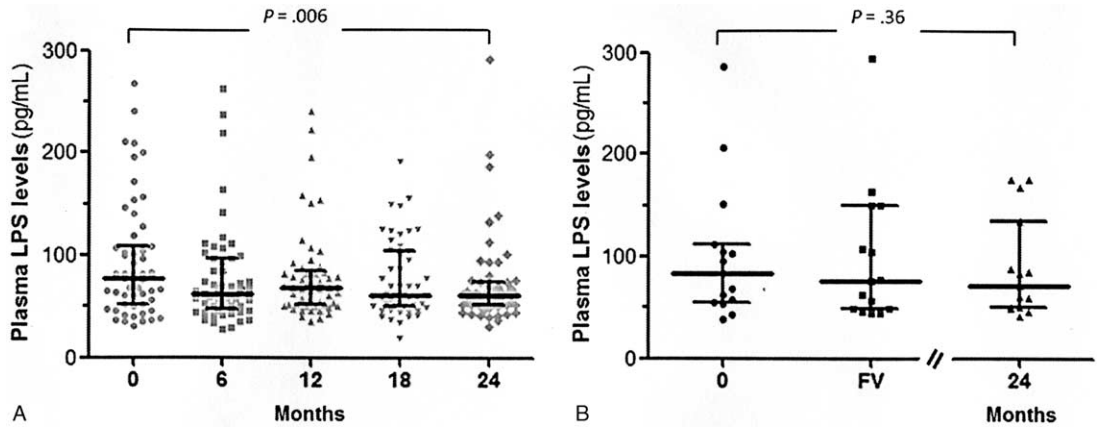

FIGURE 1. Evolution of plasma LPS levels during the 24 months of mtDRV/rtv in patients (A) without and (B) with VF. VF occurred at a median of 12 mo (IQR, 6–18; range, 6–21). IQR = interquartile range, LPS = lipopolysaccharide, mtDRV/rtv = ritonavir-boosted darunavir monotherapy, VF = virological failure.

despite of reachieving virological control (month 0, 83.6 [54.3–111.8] vs at virological failure, 75.7 [49.1–148.9] vs month 24, 72.1 pg/mL [50.8–134.6], $P = 0.76$; Figure 1B).

Both plasma 16S rDNA (month 0, 5.35 [4.97–5.79] vs month 24, 5.44 $\log_{10}$ copies/mL [4.93–6.05]; $P = 0.62$) and sCD14 levels (month 0, 10.9 [9.4–13.1] vs month 24, 10.4 pg/mL [9.1–12.8]; $P = 0.18$) were unchanged during the 24 months on mtDRV/rtv irrespective of the viral outcome. No correlations between plasma LPS and 16S rDNA or sCD14 levels were found either at baseline ($\rho = -0.185$, $P = 0.123$; $\rho = -0.112$, $P = 0.176$) or throughout the follow-up ($\rho = -0.151$, $P = 0.20$; $\rho = -0.146$, $P = 0.11$), although there were strong correlations between the baseline values of each of them and the geometric means of their values during the follow-up ($\rho = 0.749$ for LPS, $\rho = 0.671$ for 16S rDNA and $\rho = 0.485$ for sCD14 levels; $P < 0.001$).

Relationship Between IA, MT, and HIV-1 Viremia

The IA profiles of $CD4^+$ and $CD8^+$ T cells were related with the virological behavior after switching to mtDRV/rtv. During the 24 months, IA decreased in CUV patients (baseline HLA-DR^+CD38^+ $CD4^+$ T cells, 4.6% vs month 24, 4.0%, $P = 0.01$; baseline HLA-DR^+ $CD38^+$ $CD8^+$ T cells, 5.3% vs month 24: 4.4%, $P = 0.001$), and remained stable in blips and in IV patients (baseline HLA-DR^+CD38^+ $CD4^+$ T cells, 5.0% vs month 24, 4.0%, $P = 0.13$; baseline HLA-DR^+CD38^+ $CD8^+$ T cells, 5.9% vs month 24, 4.8%, $P = 0.75$). By contrast, IA increased in patients with VF (baseline HLA-DR^+CD38^+ $CD4^+$ T cells, 6.1% vs month 24, 7.1%, $P = 0.02$; baseline HLA-DR^+CD38^+ $CD8^+$ T cells, 7.4% vs month 24, 9.4%, $P = 0.06$).

Throughout the follow-up, the changes in HLA-DR^+CD38^+ $CD4^+$ and $CD8^+$ T cells were not associated to LPS or sCD14 levels, but weak correlations were found only between plasma 16S rDNA levels and HLA-DR^+CD38^+ $CD4^+$ ($\rho = 0.286$, $P = 0.07$) and $CD8^+$ T cells ($\rho = 0.331$, $P = 0.04$).

On the other hand, there were no correlations between HIV-RNA and LPS levels ($\rho = -0.085$, $P = 0.13$) or 16S rDNA levels ($\rho = 0.013$, $P = 0.82$) or sCD14 levels ($\rho = 0.013$, $P = 0.31$) during the 24 months on mtDRV/rtv. In contrast, positive correlations were found between HIV-RNA and the percentages of HLA-DR^+CD38^+ $CD4^+$ ($\rho = 0.102$, $P = 0.05$) and $CD8^+$ T cells ($\rho = 0.162$, $P = 0.01$). Finally, a multiple linear regression model showed that HIV-RNA was the only variable associated with changes in HLA-DR^+CD38^+ $CD4^+$ and $CD8^+$ throughout the follow-up ($\beta = 4.78$, $P < 0.001$ and $\beta = 2.93$, $P = 0.005$, respectively).

DISCUSSION

We have assessed MT by 2 different markers (LPS and 16S rDNA) and a surrogate marker (sCD14). As in the study by Abad-Fernández et al,[14] no correlations of bacterial 16S rDNA with either with LPS or sCD14 were found. In addition, we also failed to find a relationship between LPS and sCD14 levels perhaps because sCD14 is an activation marker for monocytes, but not a direct or specific marker of MT,[29] and stimuli other than MT could have a major influence on innate immunity activation in patients with a long time of viral suppression and immune reconstitution.[30,31]

Our results show that although plasma 16S rDNA and sCD14 levels remained stable throughout the 24 months on mtDRV/rtv irrespective of patients' viral outcomes, LPS levels decreased, but only in patients without VF. This decline was more pronounced among patients with CUV, which might suggest a continuous recovery of the mucosal immune dysfunction and/or LPS clearance mechanisms, including the clearance by Kupffer cells.[32,33] On the contrary, neither at baseline nor during the follow-up was $CD4^+$ and $CD8^+$ T cells IA related to MT markers. The only variable independently associated with a decrease in $CD4^+$ and $CD8^+$ T cells activation was an undetectable viremia throughout all the follow-up. In a completely different clinical setting, Srinivasula et al[34] also found that HIV was the primary driving force behind the activation and proliferation of most subsets of both CD4 and CD8 T lymphocytes in HIV-infected patients, whereas MT had not an important role in this proliferation.

Nevertheless, this observational study have several limitations among which are the small sample size in each group of patients and that it was a single-arm trial, without a comparator group on triple therapy. Thus, these results need to be confirmed with larger prospective clinical studies including both types of treatment. Likewise, these results could be different in earlier treatment phases or in patients with higher immunological

impairment. However, to date, there are few data on factors influencing the $CD4^+$ and $CD8^+$ T cells IA in HIV-1-infected patients on ritonavir-boosted protease inhibitor monotherapy. In this context, our results might help to clarify one of the several concerns about this maintenance strategy in patients with long-term viral suppression.

In summary, our results suggest that MT does not have a pivotal role in T-cell activation in patients with long-term viral suppression and relatively high $CD4^+$ T-cell counts. The viremia and VF are the main factors related to $CD4^+$ and $CD8^+$ T cells IA, even during mtDRV/rtv.

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