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Short communication

Microbial translocation is associated with residual viral replication in HAART-treated HIV+ subjects with <50 copies/ml HIV-1 RNA

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ABSTRACT

Background: Recent data have shown that plasma levels of lipopolysaccharide (LPS) are a quantitative indicator of microbial translocation in HIV infected individuals.
Objectives: To assess the impact of residual viral replication on plasma LPS in HAART-treated HIV+ subjects with <50 copies/ml HIV-1 RNA and to evaluate LPS changes during repeated HAART interruptions not exceeding 2-month duration.
Study design: LPS was measured in 44 HIV+ subjects at T0 (during HAART) and at day 15 of the first and fourth HAART interruption. Ten uninfected, healthy donors were studied as well. Residual plasma HIV-1 RNA was measured at T0 by an ultra-ultrasensitive method with limit of detection of 2.5 copies HIV-1 RNA/ml. Subjects with less than 2.5 copies/ml (fully suppressed – FS) were compared to those with 2.5–50 copies/ml (partially suppressed – PS).
Results: At T0, plasma LPS levels were comparable in FS and uninfected subjects, whereas in PS they were higher than in uninfected subjects ($p = 0.049$). After 4 HAART interruptions, they did not change significantly. However, LPS values were lower in FS than in PS ($p = 0.020$). An inverse correlation was found between CD4 and LPS levels ($p = 0.044$) in PS group only.
Conclusions: A reduced degree of microbial translocation was seen in subjects with a more complete suppression of viral replication. Repeated HAART interruptions had no significant impact on plasma LPS levels.

1. Background

Recent data have shown that the microbial translocation consequent to the impairment of the gastrointestinal barrier plays an important role in the generalized immune activation that is typical of HIV disease.[1,2] The degree of microbial translocation can be assessed by measuring plasma levels of bacterial byproducts such as lipopolysaccharide (LPS) and it has been associated to HIV progression.[3] In fact, increased LPS levels have been found in HIV infected subjects respect to HIV uninfected. Highly active antiretroviral therapy (HAART) induces a decrease in microbial translocation,[4,5] which nevertheless remain higher than in healthy individuals.[6]

2. Objectives

The aim of the present study was to assess if different degrees of viral suppression in HAART-treated subjects with <50 copies/ml of HIV-1 RNA influence the amount of circulating LPS. In the same cohort of subjects, LPS changes were analyzed during intermittent HAART.

3. Study design

Study population consisted of subjects enrolled in the arm B of the Istituto Superiore di Sanità-Pulsed Antiretroviral therapy (ISS-PART) clinical trial (5 treatment interruptions of 1-, 1-, 2-, 2- and 3-month duration, each one separated by 3-month therapy),[7] and selected according to a viral load <50 copies at study entry and the availability of 3 plasma samples taken at T0 (during HAART) and at first and fourth treatment interruption. Patients with HBV or HCV coinfection were excluded from this study. On the basis of HIV-1 RNA plasma levels patients were dichotomized into "fully suppressed" (FS), with less than 2.5 copies/ml, and "partially suppressed" (PS), with more than 2.5 copies/ml.

Abbreviations: LPS, lipopolysaccharide; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy.

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LPS levels were determined in frozen ethylenediamine tetraacetic acid (EDTA) plasma samples diluted 1:1000 with endotoxin-free water using a commercial available Limulus Amebocyte assay (Cambrex Bioscience, Walkensville, Maryland, USA), according to the manufacturer instructions. Plasma samples were heated at 70 °C for 20 min to inactivate inhibitory plasma proteins. Results were expressed in EU/ml. Samples were tested at T0 and 2 weeks after the first (T0 + 15) and fourth interruption at month 13 (T 13 + 15). Plasma LPS was also measured in 10 uninfected, healthy donors.

At T0, plasma HIV-1 RNA below 50 copies/ml was quantified by an ultra-ultra sensitive method based on a modified Amplicor HIV-1 Monitor test version 1.5 (Roche Molecular Systems, Branchburg, New Jersey, USA), with a limit of detection of 2.5 copies/ml as previously described.[8]

Descriptive results are presented as medians and percentages. For intergroup comparisons, Mann–Whitney *U*-test, Fisher's exact test and Wilcoxon signed rank test were used as appropriate. The Spearman's rank coefficient was used for determining correlations. All analyses were performed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL US).

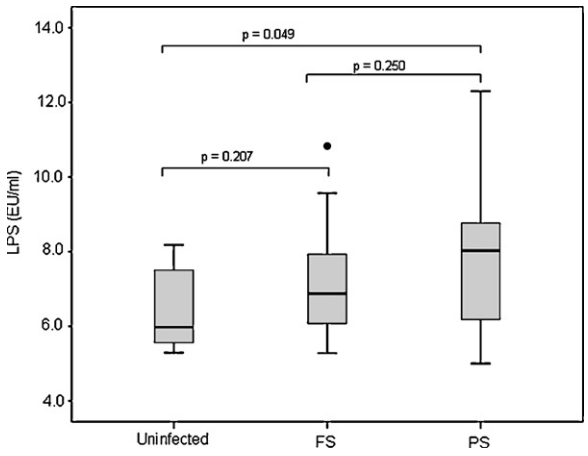

Fig. 1. Circulating lipopolysaccharide (LPS) levels in uninfected and HIV infected subjects under HAART with <50 copies/ml HIV-1 RNA for at least 6 months. Group FS (HIV-1 RNA below 2.5 copies/ml); group PS (HIV-1 RNA between 2.5 and 50 copies/ml).

4. Results

Forty-four subjects, all on first line HAART, were studied. Their median value of HIV-1 RNA was 4.17 copies/ml; 19/44 (43.2%) were FS and 25/44 (56.8%) were PS (Table 1).

Median levels of plasma LPS were: 7.4 EU/ml (range 5.0–12.3 EU/ml) in the HIV infected and 5.97 EU/ml (range 5.29–8.18 EU/ml) in the 10 uninfected subjects ($p = 0.070$). When the two groups, FS and PS, were considered, the former only had LPS values comparable to those of uninfected subjects ($p = 0.207$), whereas higher values of LPS were seen in PS subjects respect to the uninfected ones ($p = 0.049$). No differences were found between the two HIV infected groups ($p = 0.250$) (Fig. 1).

Two weeks after the first HAART interruption (T0 + 15), viral rebound >50 copies/ml occurred in 92% of PS and 63% of FS ($p = 0.027$), whereas no significant changes were observed in CD4 and CD8 cell count. LPS plasma levels were similar in the groups (FS: 7.72 EU/ml, range 5.0–14.7; PS: 7.73 EU/ml, range 5.11–12.6), with no significant changes respect to the T0 values.

The same patients were longitudinally monitored. Two weeks after the fourth HAART interruption (T 13 + 15) viral rebound occurred in 92.3% of PS and 68.8% of FS ($p = 0.183$). LPS plasma levels were significantly lower in FS (5.16 EU/ml, range 4.53–12.03) than in PS (7.07 EU/ml, range 4.64–11.56) ($p = 0.029$), although there were no significant changes from T0 ($p = 0.078$) in either group. The magnitude of viral rebound and LPS plasma levels were positively correlated ($R = 0.608$, $p < 0.001$, Fig. 2) in both groups. A significant reduction in CD4 cell count from T0 ($p = 0.002$). and an inverse correlation between CD4 and LPS levels ($R = -0.525$, $p = 0.044$) were observed in PS group (Fig. 3).

5. Discussion

In our study we investigated the potential relationship between residual HIV-1 viremia below 50 copies/ml and LPS in HAART responders. By arbitrarily dichotomizing subjects into "Fully" or "Partially" suppressed on the basis of HIV-1 RNA levels below 2.5 or between 2.5 and 50 copies/ml, we found that only the former subset had levels of LPS comparable to those seen in the uninfected controls.

Brenchley et al.[4] found that HAART decreases LPS but does not totally abrogate its production, and did not see correlations between LPS plasma levels and viral load in HAART-treated subjects. In a large population of HAART-treated patients, Jiang et al.[6] measured plasma levels of bacterial DNA (16S rDNA) as a marker of microbial translocation and found a strong association between T cell activation (and lower CD4 T cell restoration) and higher levels

Table 1
Study population. Values are expressed as median and range or percentage.

	All ($n = 44$)	FS[a] ($n = 19$)	PS[b] ($n = 25$)	*p*-Values
Age (years)	37 (26–71)	37 (28–71)	37 (26–63)	0.686
Sex (% of males)	68.2	57.9	76.0	0.327
HAART duration (months)	22 (12–44)	26 (13–36)	21 (12–44)	0.493
HAART regimen				
PI-based	12 (27.3%)	5 (26.3%)	7 (28.0%)	
NNRTI-based	29 (65.9%)	13 (68.4%)	16 (64%)	0.950
3 NRTI	3 (6.8%)	1 (5.3%)	2 (8%)	
Ultra-ultra sensitive HIV-1 RNA (copies/ml)	4.17 (2.5–49)	<2.5	8.6 (2.5–49)	<0.001
CD4 cell count				
Cells/μl	729 (370–1365)	728 (370–1062)	731 (418–1360)	0.286
%	35 (18–57)	36 (18–56)	34 (19–57)	0.893
CD8 cell count at time 0				
Cells/μl	766 (391–1467)	710 (412–1260)	856 (391–1467)	0.415
%	39 (24–63)	39 (29–60)	39 (24–63)	0.864

[a] Subjects with HIV-1 RNA plasma levels <2.5 copies/ml.
[b] Subjects with HIV-1 RNA plasma levels between 2.5 and 50 copies/ml.