

# Lack of Effect of Short Viremic Episodes on LPS Plasma Levels in Chronically HIV-1-infected Subjects

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## ABSTRACT

**Background:** Previous reports showed the presence of increased levels of lipopolysaccharide (LPS) in HIV-1 infected progressors. We investigated the effect of short treatment interruptions (TI) on plasma LPS levels in chronically HIV-1 infected patients.

**Methods:** A total of 76 subjects participated in this study (50 HIV<sup>+</sup> and 26 healthy). 9/50 HIV<sup>+</sup> subjects were viremic non-ART-treated patients, while 41/50 were chronically suppressed patients on ART (n=3 drugs, CD4 count>400, HIV-1 RNA<500 for 8 months, <50 at recruitment) followed for 40 weeks on continuous ART (20/41, non-TI group) or undergoing two TIs [21/41: a 6 week and an open-ended TI (TI group)]. T cell activation (CD8<sup>+</sup>/CD38<sup>+</sup>, CD8<sup>+</sup>/HLA-DR<sup>+</sup> and CD3<sup>+</sup>/CD95<sup>+</sup>) by same day whole blood flow cytometry and plasma levels of LPS and IgM endotoxin-core antibodies (EndoCAB) by ELISA were measured at: a) a single visit (healthy and viremic non-ART-treated), b) sequential visits including follow-up during short-term 6 week and >20 weeks of therapy interruption (TI group) and c) beginning and day 40 of follow-up on continuous ART (non-TI group). Variables levels between healthy and viremic were compared by t-test or the Wilcoxon/Kruskal-Wallis test (Rank Sums) depending on the data distribution. Correlations between variables were assessed using Spearman or pairwise correlation tests. All statistics were performed with JMP4.

**Results:** LPS plasma levels were significantly lower in healthy patients when compared to steady-state viremic patients for >20 weeks off therapy (n=9, p<0.0001). As a result of a TI-related acute viremia, increased T cell activation and a negative association between plasma LPS levels at the same time point as well as with EndoCAB change during start and end of the 6 week TI (p=0.0152, Spearman's Rho=-0.6124 and p=0.0204, correlation=-0.502 respectively) were observed. In addition, comparisons between the change in EndoCAB and LPS from time on therapy to a subsequent steady viremic time-point >12 weeks after TI showed a negative association (p=0.0073, correlation=-0.8514). Finally, activation markers changes during acute viremia or CD4 count were not associated with LPS or EndoCAB.

**Conclusions:** These data showing increased LPS levels in prolonged viremia compared to healthy subjects, and a negative association between EndoCAB and LPS change during TI suggest an active association between HIV and microbial translocation in HIV-1 infected patients upon long-term viremia.

## INTRODUCTION

Within the first weeks of HIV-1 infection a breach in the integrity of mucosal immune system occurs, which is characterized by a rapid depletion of the bulk of CD4 primarily localized in the gastrointestinal tract (1), along with a damage of the intestinal epithelial microenvironment (2). This eventually results in translocation of microbes and/or microbial products without overt bacteremia (microbial translocation) (3).

In several conditions (graft-versus-host disease, inflammatory bowel disease, invasive gastrointestinal surgery) (4) the degree of microbial translocation is determined by assessment of plasma levels of lipopolysaccharide (LPS), a major component of Gram-negative bacterial cell wall and a potent immunostimulatory product (5). LPS activity can be neutralized by naturally occurring immunoglobulin-M (IgM), IgA and IgG antibodies to the LPS core oligosaccharide (endotoxin-core antibodies, EndoCAB), that are probably produced by T-dependent B cells and can be measured in plasma (6).

A shown by Douek et al (7), introduction of antiretroviral therapy (ART) in HIV-1 infection results in reduction in plasma LPS levels. This together with the finding by the same group that LPS levels are increased in HIV-1 infected progressors, suggest that HIV replication plays a central role in perpetuating microbial translocation and directly contributes to systemic immune activation in chronic HIV infection. The effect, if any, of short viremic episodes in microbial translocation remains unknown. Based on these observations we hypothesized that therapy interruption (TI)-mediated short viremic episodes may increase the plasma levels of LPS above the levels observed in the course of continuous ART, and investigated the effect of short viremic episodes on changes in plasma LPS in chronically HIV-1 infected patients.

## SUBJECTS, MATERIALS & METHODS

Cryopreserved plasma samples from 76 subjects (50 HIV<sup>+</sup> and 26 healthy) were used for this study. 9/50 HIV<sup>+</sup> subjects were viremic non-ART-treated patients, while 41/50 were chronically suppressed patients on ART (≥3 drugs, CD4 count>400 cells/ml, a nadir CD4 ≥100 cells/ml, plasma HIV-1 RNA<500 copies/ml for 6 months and <50 copies/ml at recruitment) participating in a parent study (8).

Four sets of samples were analyzed: a) 26 healthy (Group 1) and 9 HIV<sup>+</sup> viremic ART-naïve subjects (Group 2) analyzed at a single visit; b) 21 subjects analyzed at study entry (on ART, <50 copies/ml), as well as during sequential visits including a short-term 6 week and/or >20 weeks of therapy interruption (TI) on ART (<50 copies/ml) and a viremic time point during TI analyzed each time respectively (Group 3); c) 20 subjects analyzed at beginning and end of 40 weeks follow-up on continuous ART (<50 copies/ml) (Group 4).

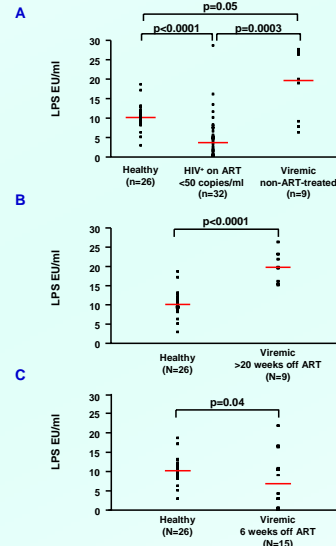
T cell activation (CD8<sup>+</sup>/CD38<sup>+</sup>, CD8<sup>+</sup>/HLA-DR<sup>+</sup> and CD3<sup>+</sup>/CD95<sup>+</sup>) was assessed, using whole blood flow cytometry at the time of blood collection. Plasma levels of LPS and IgM endotoxin-core antibodies (EndoCAB) were determined by Limulus Amebocyte assay (Cambrex) and ELISA (Cell Sciences) respectively according to the manufacturer's specifications. All measurements were based on the average of duplicate samples. Lower limits for LPS and EndoCAB were 0.1 EU/ml and 0.054 MMU/ml respectively.

The data are described as medians, 25<sup>th</sup> and 75<sup>th</sup> percentiles for all variables. Variable distributions were analyzed for normality using the Shapiro-Wilk W test (p>0.05). Variables levels between healthy and viremic were compared by t-test or the Wilcoxon/Kruskal-Wallis test (Rank Sums) depending on the data distribution. Between-time points comparisons were performed using non-parametric Wilcoxon Sign-Rank test or paired t-tests depending on the data distribution. Correlations between variables were assessed using Spearman or pairwise correlation tests. Descriptive analysis and statistical tests were performed using JMP4 (SAS Institute).

## Differential effect of Long versus Short-term HIV-1 Replication on LPS Plasma Levels

Long-term viremia, in contrast to short-term viremia, resulted in increased plasma LPS levels when compared to healthy controls subjects

LPS plasma levels were significantly higher in ART-naïve HIV-1 patients (n=9, median plasma HIV-1 RNA=19601 (11545, 69954)) and in steady-state viremic HIV-1 patients undergoing a TI >20 weeks (n=9, median weeks=19 (12.5, 35), median plasma HIV-1 RNA=43748 (23192, 101044)) when compared to healthy subjects (n=26, p=0.05, Figure 1A; p<0.0001, Figure 1B respectively) or ART-suppressed (<50 copies/ml) HIV-1 patients (n=32, p=0.0003 Figure 1A; n=17, p<0.0001 respectively). To our surprise, LPS levels were lower in ART-suppressed (<50 copies/ml) HIV-1 patients (n=32) when compared to healthy subjects (n=26, p<0.0001, Figure 1A). In addition, LPS levels were lower in HIV-1 patients undergoing a 6 week TI (n=15, median plasma HIV-1 RNA=10745 (2527, 61874)) when compared to healthy subjects (n=26, p=0.04, Figure 1C).

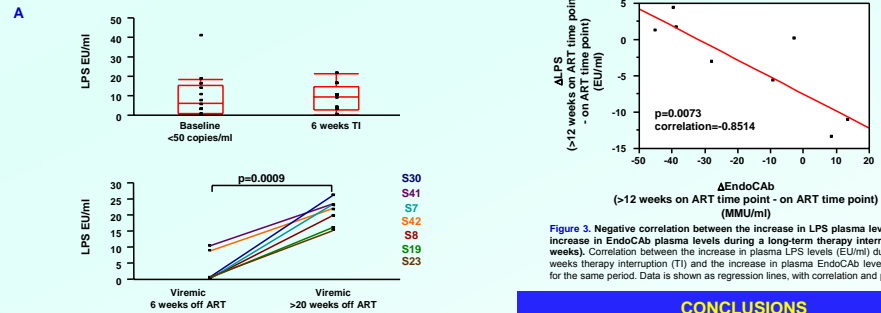


**Figure 1. Increased plasma LPS levels during long-term viremia.** Plasma LPS levels are shown for: A, healthy, ART-suppressed and ART-naïve HIV-1 subjects; B, healthy and HIV-1 subjects at long-term viremia (>20 weeks therapy interruption, TI); C, healthy and HIV-1 subjects at short-term viremia (6 weeks TI). Based on the data distribution median is shown in Panel A, while mean is shown in panels B and C. Significant p values are shown on the top of each graph.

## Association Between Viremia, LPS & EndoCAB

Negative association between plasma levels of LPS & EndoCAB during treatment interruption-mediated viral rebound

As expected, a 6 week TI in Group 3 resulted in an increase in immune activation (data not shown). No difference was observed between LPS levels during a 40 week follow-up on ART (Group 4, data not shown) or at the beginning and end of the 6 week TI (Group 3, Figure 2A top panel). Interestingly LPS levels were higher at >20 weeks TI when compared to levels at the end of a 6 week TI (n=7, p=0.009, Figure 2A bottom panel). During the 6 week TI in Group 3, a negative association of plasma HIV-1 RNA at week 6 with LPS plasma levels at the same time point (p=0.0152, Spearman's Rho=-0.6124, Figure 2A), as well as with EndoCAB change from the start to the end of the 6 week TI (p=0.0204, correlation=-0.502, Figure 2B) were observed. The negative association of plasma LPS levels and viremia, as well as the decrease of EndoCAB plasma levels in the presence of short-term (6 weeks) viremic episode could be attributed to EndoCAB binding to LPS. This was further suggested by a negative association observed between the change in EndoCAB and LPS from time on therapy to a subsequent steady viremic time-point >12 weeks after TI (p=0.0073, correlation=-0.8514, Figure 3). No association between activation markers changes during acute viremia or CD4 count with LPS or EndoCAB were found (data not shown).

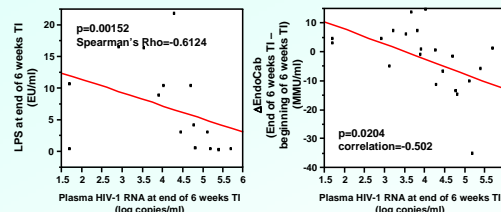


**Figure 3. Negative correlation between the increase in LPS plasma levels and the increase in EndoCAB plasma levels during a long-term therapy interruption (>12 weeks).** Correlation between the increase in plasma LPS levels (EU/ml) during >12 weeks therapy interruption (TI) and the increase in plasma EndoCAB levels (MMU/ml) for the same period. Data is shown as regression lines, with correlation and p values.

## CONCLUSIONS

HIV-1 replication is associated with microbial translocation in ART-naïve and in HIV-1 infected patients undergoing long-term therapy interruption

A negative correlation between LPS and EndoCAB following therapy interruption suggests that rises in LPS are observed after long-term therapy interruption and not during short-term therapy interruption because of: a) drop in EndoCAB levels as a result of its binding to LPS or decreased functionality of B cells, b) positive effect of viral replication or length of the interruption on microbial translocation, and c) increased microbial translocation above levels able to be affected by EndoCAB



**Figure 2. Negative correlation between plasma HIV-1 RNA at 6 week ART interruption and both LPS plasma levels and EndoCAB change during a 6 week therapy interruption.** A, LPS levels (EU/ml) in HIV-1 subjects are shown at suppression and at 6 week therapy interruption (TI) (top panel), as well as at 6 week and >20 week TI (bottom panel); B, Correlation between plasma LPS levels (EU/ml) and plasma HIV-1 RNA (log copies/ml) at 6 week TI (left panel); C, Correlation between the change in plasma EndoCAB levels (MMU/ml) during a 6 week TI and plasma HIV-1 RNA (log copies/ml) at week 6 (right panel). In the top part of panel A data is shown as inter-quartile box plot (median and outliers), while the bottom part of panel A shows LPS level for 7 patients. In panel A, significant p values are shown on the top of each graph. Data in panel B is shown as regression lines, with correlation and p values.

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