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Increased Gut Microbial Translocation in Human Immunodeficiency Virus-Infected Children Persists in Virologic Responders and Virologic Failures Following Antiretroviral Therapy

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Abstract

Background—Gut microbial translocation (MT) is considered a major cause of immune activation (IA) and failure of immune reconstitution in HIV infection. This study investigated the relationship of virus replication, IA, CD4 counts and MT in HIV-infected children.

Methods—Lipopolysaccharide (LPS), bacterial 16S ribosomal DNA (16SrDNA) and soluble CD14 (sCD14) levels were determined in prospectively collected, stored plasma samples from PACTG 338, a 48 week study initiated in 1997 to compare efficacy of dual nucleosides with a ritonavir-containing regimen. Results of MT were correlated with study data for T cell IA, plasma virus load (VL) and CD4 counts in 85 HIV-infected children (ages 2–17 yrs) designated virologic responders (VR) or virologic failures (VF) at week 44 based on a cut-off of 400 HIV RNA copies/mL.

Results—Levels of plasma LPS, 16SrDNA and sCD14 were increased in comparison to HIV-uninfected controls and did not decrease at week 44 even in VR patients. T cell IA was correlated with VL and sCD14 at entry and with 16SrDNA and sCD14 at week 44 in total patients and in the VF group. Changes in 16SrDNA correlated with changes in IA and negatively with changes in CD4 counts. 16SrDNA was correlated with sCD14 but not with LPS.

Conclusion—MT persists after 44 weeks of ART in VS and VF patients. In VF, 16SrDNA exhibited relationships to monocyte and T cell IA and CD4 counts but not with VL, suggesting a dominant role for MT in disease pathogenesis in HIV-infected children.

Keywords

microbial translocation; immune activation; HIV infection; lipopolysaccharide; 16SrDNA

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Potential conflict of interest: none

Immune activation is a major component of disease progression in chronic HIV infection and leads to excessive destruction of CD4 T cells by apoptosis, uncontrolled virus replication in activated CD4 T cells and immune exhaustion. Manifestations of immune activation include polyclonal B cell hypergammaglobulinemia, increased T cell turnover, increased frequency of activated T cells and increased levels of proinflammatory cytokines and chemokines [1]. Mechanisms of immune activation have been difficult to pinpoint and were initially attributed to direct and indirect effects of HIV infection [2-4]. Following identification of the gut as a major site of HIV replication with loss of CD4 T cells, Brechley et al brought attention to the potential role of translocation of microbial products from the gut into the systemic circulation as a causative factor in immune activation in chronically HIV-infected adults [5]. Subsequently, it was shown that loss of TH-17 cells and structural damage to the gut epithelium result in MT and IA [6, 7].

MT involves passage of gut derived microbes and/or microbial products into the systemic circulation without overt bacteremia. MT is assessed by quantifying plasma levels of lipopolysaccharide (LPS), which is a major component of gram-negative bacteria cell walls, or plasma levels of 16SrDNA, which represents the conserved sequences of bacterial DNA fragments common to most bacteria and LPS core antigen [8]. Elevated LPS levels in plasma are indicative of increased intestinal permeability and occur after invasive gastrointestinal surgery [9], in graft-versus-host disease and in inflammatory bowel disease [10]. In adults with chronic HIV infection, levels of plasma LPS in “progressors” are increased as compared to non-progressors or uninfected individuals, and have been correlated with activated CD8 T cells expressing increased CD38 molecules and with sCD14, indicating monocyte activation [5]. Monocytes and macrophages express membrane CD14 and secrete soluble CD14 (sCD14) upon activation. Measurement of plasma sCD14 provides evidence for direct chronic LPS stimulation of monocytes and macrophages *in vivo*. More recently, LPS in plasma was found to be correlated with 16SrDNA, CD8 T cell immune activation and with sCD14, a marker of monocyte activation [5, 11-13]. In patients on potent antiretroviral therapy (ART), persistent IA and ongoing evidence of microbial translocation, despite virologic control, has been linked to impaired CD4 T cell reconstitution [14]. In HIV-infected children data on MT is limited and has been derived mainly from the LPS assay. Interestingly, two studies in ART naïve children documented high LPS levels which did not correlate with immune activation and LPS levels did not decrease after potent ART even in patients who controlled virus replication [15, 16]. These findings differ from data in HIV-infected adults and also differ by their lack of correlation with CD4 T cell immune reconstitution. In both pediatric studies, active thymus output was credited for replenishing the CD4 T cells.

Because IA is such an important aspect of HIV disease, investigation of IA in different clinical scenarios could enhance our understanding of underlying mechanisms. The present study investigated a set of prospectively collected plasma samples from a completed PACTG study (P338) in which ART experienced, immunologically stable but viremic HIV infected children were given a protease inhibitor (PI) based regimen or dual nucleosides. As the outcome on HIV virus burden and T cell immune activation was known [17], we tested the hypothesis that gut microbial translocation is a major stimulus for immune activation that persists after control of virus replication. In the present study, we show that gut MT persists after control of virus replication and is associated directly or indirectly with generalized immune activation. Further we found that IA was linked to MT rather than virus load in patients who failed to control virus replication on ART.

MATERIAL AND METHODS

Study design

Patients—The study was performed using plasma samples that had been collected and stored at -80°C during the conduct of Pediatric AIDS Clinical Trials Group (PACTG) 338. This randomized, phase II clinical trial was conducted between February 1997 and April 1977 in antiretroviral therapy-experienced, protease inhibitor (PI)-naïve, HIV-infected, clinically stable children aged 2–17 years. The main study enrolled 298 HIV-infected children. The treatment arms consisted of a dual-nucleoside analogue regimen (zidovudine plus lamivudine) which was compared with a regimen containing the PI ritonavir in combination with dual or single nucleosides. Plasma virus load was determined by the NucliSens Assay (Organon Teknika, Durham, NC), and had a lower limit of quantification of 400 copies/mL [17, 18]. The study demonstrated that patients on the PI regimen had a higher frequency of attaining sustained virologic suppression to < 400 HIV RNA copies/mL [18]. An immunology substudy evaluated immune activation markers HLA-DR and CD38 on CD4 and CD8 T cells in 147 patients by flow cytometry in select PACTG immunology laboratories using a standardized protocol, which demonstrated a decrease in T lymphocyte immune activation at week 44–48 in PI containing arms [17]. The viral load (VL) and flow cytometry data from the previously published study [17, 18] was used to establish correlations with the plasma assays performed in the present study. The 85 children included in this manuscript were the ones for whom samples were available.

Laboratory Assays for plasma samples—For the present study, we examined plasma samples at week 0 and week 44 or 48 from 85 children from the immunology substudy. All week 44/48 samples are referred to as week 44. Microbial translocation markers (LPS, 16SrDNA) and monocyte activation marker (sCD14) were measured in plasma that had been stored at -80°C and shipped to the testing laboratory in dry ice. Plasma LPS was assayed only in those samples in which the anticoagulant was EDTA ($n=70$). The other biomarkers, 16SrDNA ($n=76$) and sCD14 ($n=85$), were determined in all available samples.

Plasma LPS analysis—Ten microliters of plasma was diluted 1:10 in endotoxin-free water, and heat-inactivated at 85°C for 15 min to inactivate inhibitory plasma proteins. LPS levels were measured in plasma by the use of the limulus amoebocyte lysate (LAL) chromogenic endpoint assay (Lonza Group Ltd, Allendale, New Jersey, USA) according to the manufacturer's recommendations and calculated in relation to an *Escherichia coli* endotoxin standard provided with the assay, after background subtraction [5]. Results of LPS measured were expressed in pg/mL.

16SrDNA quantitation in plasma—DNA was extracted from 200 μL of plasma by use of the DNeasy Blood and Tissue Kit (Qiagen INC, CA, USA). DNA purity and concentration were determined by nanodrop spectrophotometer (Thermo scientific, DE, USA). Real time fluorescence detection was used with the ABI PRISM 7700 sequence detector (Perkin Elmer Applied Biosystems, CA, USA) to quantify the bacterial 16SrDNA level in plasma. DNA was amplified in a reaction mixture consisting of 2 μL of 10 $\times$ PCR buffer, 3.5 mmol/L MgCl_2 , 0.2 mmol/L dNTPs, 0.5 $\mu\text{mol/L}$ forward (8F: 5'-AGT TTG ATC CTG GCT CAG-3') and reverse (515R: 5'-GWA TTA CCG CGG CKG CTG-3') primers, 0.32 $\mu\text{mol/L}$ probe (338P: 5'-FAM-GCT GCC TCC CGT AGG AGT-BHQ1-3'), 0.75 U of Taq polymerase and 5 μL of DNA. The reaction conditions for amplification of DNA were 95°C for 5 min, followed by 45 cycles at 95°C for 15 seconds and at 60°C for 1 min. Real time PCR was performed in duplicates for each standard dilution and sample and mean CT value of the duplicate PCRs was determined and used for the calculations. A standard curve was created from serial dilutions of plasmid DNA containing known copy numbers of the

template obtained from Dr Daniel Douek at the Vaccine Research Centre at NIH who also provided the sequence of the primers and probe for the PCR. Copy numbers of the samples were calculated from the standard curve by interpolation [11]. Results were expressed as 16SrDNA copy number/ μ l plasma.

Plasma sCD14 analysis—Monocytes and macrophages, expressing membrane CD14, secrete soluble CD14 (sCD14) upon activation. Measurement of plasma sCD14 provides evidence for direct chronic LPS stimulation of monocytes and macrophages *in vivo*. Plasma levels of sCD14 were quantified by Human sCD14 Immunoassay (R&D Systems, Minneapolis, Minnesota, USA). Ten microliters of plasma was diluted 200-fold by addition of 1990 μ l calibrator diluent and assayed in duplicate. Results of sCD14 were expressed in pg/ml.

Statistical analysis—Differences in the variables between week 0 and week 44 were analyzed using paired signed ranked test and student's paired 2-tailed t-test. When we used paired comparison to evaluate the changes of different markers between weeks 0 and 44, only the subjects who had the marker values at both time points were included. Correlations between two variables were done using the Spearman correlation and linear regression. The treatment arms were not taken into consideration. Data for LPS, 16SrDNA and sCD14 was analyzed in relation to CD4 counts, VL and T cell activation markers.

RESULTS

Changes in plasma virus load, CD4 counts and T cell immune activation following ART

Characteristics of the subset of patients whose samples were analyzed for one or more plasma markers in the present study are shown in table 1. Based on the virologic outcome at week 44, patients were designated as virologic responders (VR, VL < 400 copies) and virologic failures (VF, VL > 400 copies). The VL and CD4 counts at baseline were similar between VR and VF subgroups by 44 weeks of ART; a significant gain in CD4% was noted in VR ($p=0.005$) but not in VF groups. CD4 and CD8 T cell activation markers (HLA-DR +CD38+), which were similar at baseline between VF and VR groups, decreased in the patient population as a whole (CD4; $p=0.02$, CD8; $p=0.004$) and in VR (CD4; $p=0.03$, CD8; $p=0.002$) but not in VF patients. Thus, greater T cell activation was associated with virologic failure and with failure of CD4 T cell reconstitution.

Changes in plasma levels of microbial translocation following ART

Plasma LPS and 16SrDNA were significantly higher at study entry than values of healthy controls in our laboratory (Data not shown). These two MT markers did not correlate with each other (Fig 1A), and changes in LPS from baseline to week 44 were also not correlated with the changes of 16SrDNA. At week 44, LPS showed a slight increase (Fig 1B) and 16SrDNA remained elevated (Fig 1C), but there was no difference in LPS or 16SrDNA between weeks 0 and 44 in VR or VF patients (not shown). The monocyte/macrophage activation marker sCD14, which reflects LPS bioactivity *in vivo*, was also elevated at study entry compared to healthy controls and remained elevated at week 44, with a significant increase in the total patients and in the VF subgroup (Figs. 1D and 1E, respectively). Changes of sCD14 from baseline to week 44 were correlated with the changes of LPS ($p=0.01$) but were not correlated with the change in 16SrDNA.

Correlation of immune activation with MT products

Interestingly, both LPS (Fig 2A) and 16SrDNA (Fig 2B) were correlated with sCD14 in the patient cohort at baseline. At week 44, 16SrDNA was still correlated with sCD14 in total patients (Fig 2C) and in VF patients (Fig 2D), but not in the VR patient group (Fig 2E).

CD4 and CD8 T cell immune activation did not correlate with LPS or 16SrDNA at baseline (not shown). At week 44 however CD4 and CD8 T cell activation manifested a strong correlation with 16SrDNA (Figure 3A, 3B) in total patients and in the VF group (Fig 3C, 3D) but not in VR (Fig 3E, F). Change in CD4 and CD8 activation from baseline to week 44 was also positively correlated with change in 16SrDNA ($p<0.05$).

Levels of sCD14 correlated with activated CD4 and CD8 T cells at baseline and at week 44 (Fig 4A-D) and this correlation was evident only in VF (Fig 4E, F) and not in the VR group at week 44 (Fig 4G, H).

Relationship of T cell immune activation with VL

We examined the relationship of CD4 and CD8 immune activation markers at baseline, at week 44 and the change from baseline with virus load. At baseline, the CD4 and CD8 T cell immune activation (DR+CD38+) was positively correlated with viral load (Fig 5A, B) in the total patient population. At week 44, activated CD4 and CD8 T cells did not correlate with VL in total patients (Fig 5C, D), the VF group (Fig 5E, F) or in the VR group (not shown).

Relationship of VL with MT

We also examined the relationship of HIV plasma virus load with each plasma MT marker at baseline, week 44 and change over week 44. Virus load did not show a correlation with LPS or 16SrDNA, but was correlated with sCD14 at baseline. At week 44 none of the MT markers correlated with VL or change in VL. In VR and VF, none of the MT markers correlated with VL at either baseline or at week 44.

Correlation of CD4 counts with MT products

Both absolute CD4 counts and CD4% were correlated negatively with LPS at week 0 (Table 2) but not with any other MT marker, whereas 16SrDNA showed a negative correlation with change in absolute CD4 count at week 44 (Table 2). CD8 counts did not show a correlation with any MT marker. CD4 T cell frequency (% CD4 T cells) showed a gain in association with a decrease in IA, but were not correlated with T cell activation even in VF at week 44 in this patient cohort.

DISCUSSION

Gut microbial translocation in HIV-infected adult populations is now well established but the relationship of gut microbial translocation with immune activation and with disease progression in different clinical scenarios is not well understood [5, 19-24] and data are limited for children with perinatally acquired chronic HIV infection [15, 16]. The present study examined prospectively collected plasma samples from an older PACTG study, P338 [18], in which the participants were a somewhat unique population of clinically stable, ART-experienced children who switched from a suboptimal regimen to a more potent protease inhibitor regimen. The results point to continued microbial translocation over a 44 week follow up with an association of the 16SrDNA with T cell and monocyte immune activation in virologic failures and a negative correlation with change in CD4 counts. The contribution of replicating virus to the immune activation was evident mainly at study entry. Our data provides further evidence for the dominant role played by products of MT from the damaged gut in HIV disease pathogenesis and its influence on disease progression.

We observed that MT products LPS and 16SrDNA persisted through the 44 weeks of follow-up even in patients who were classified as virologic successes on the ART regimen they were on. We do not consider the MT to be related to treatment drugs. There is no known direct effect of RTV on bacterial translocation (LPS, 16SrDNA) or monocyte/

macrophage activation, and both groups received nucleoside reverse transcriptase inhibitors. The gastrointestinal tract is the most prominent early site of HIV replication, with rapid and extensive depletion of CCR5+CD4 T cells occurring within days of primary HIV infection. The loss of protective immune mechanisms against gut microbes due to loss of Th17 CD4 T cells and damage to the gut enterocytes is believed to cause breaks in the mucosal epithelial barrier resulting in translocation of microbial products into the circulation [6, 25-27]. HIV DNA persists in the gut of HIV-infected patients who achieve suppression of viral replication in plasma [28]. Although potent ART given early in the disease can lead to gut CD4 T cell reconstitution [14], the time for the gut to heal following initiation of ART in chronic HIV infection is unknown. Similar to our observations, previous reports of HIV infected children show that plasma LPS levels did not decrease following ART [15, 16]. HIV-infected adults that achieve suppression of viral replication also continue to show significantly higher levels of LPS and activated T cells when compared to healthy controls [5, 23]. The cumulative data indicates that the process of gut healing in chronic HIV infection is slow in most situations and that MT will persist for an undefined period after virus load in plasma has been suppressed.

In children with perinatal HIV infection, HIV-induced gut pathology may be compounded early in life due to physiological immaturity of the gut. It was recently demonstrated that HIV-infected and HIV-uninfected infants both have increased gut permeability resulting in increased MT [15]. As the children in this study were older, we do not expect gut immaturity as a contributing factor for MT. A shortcoming of this study is the lack of age-matched HIV-uninfected controls, as the controls in this study were adults. We do not know if factors related to differences in gut microbiota in childhood and adulthood independent of HIV disease may also play a role and delay gut healing in children despite virologic control. Overall, the findings in this cohort of children with perinatal HIV infection suggests that HIV-induced gut damage persists beyond infancy, but the MT is likely to be multifactorial in its genesis.

Plasma LPS is a good quantitative indicator of MT. We found higher plasma level of LPS in HIV-infected children at baseline compared to the reported levels in infants and adult healthy controls [9,10]. In this study we also quantified 16SrDNA, which represents the conserved sequences of bacterial DNA fragments specific to LPS core antigen. There are only a few reports incorporating both LPS and 16S evaluation in adults [11, 22]. An unexpected finding was that LPS and 16SrDNA were not correlated with each other. One possible explanation for this observation could be that the products being measured were affected due to the prolonged sample storage time of more than 10 years. We consider this unlikely as our samples were maintained under strict freezing conditions and were not subjected to freeze-thaw cycles that can degrade 16SrDNA [29]. It is also conceivable that the microbiome may have changed in the course of treatment leading to fewer LPS-bearing organisms following immune reconstitution. Alternatively, 16SrDNA may, in fact, be a more sensitive indicator of MT than LPS, given that it correlated with T cell and monocyte activation, particularly in virologic failures at week 44 whereas LPS did not.

A major aim of this study was to assess the relationship of MT to IA, as there is general agreement that IA is detrimental, is associated with disease progression, and is propagated by it. The role of virus products versus MT products in causing IA has also been a subject of considerable debate. At study entry, the data were not clear cut. T cell IA correlated with virus load but not to either of the two MT products, LPS or 16SrDNA. However, this does not imply that MT was not involved because T cell IA was correlated with sCD14 which results from LPS driven TLR4 activation of monocytes and macrophages [21], arguing for a role for MT in contributing to the IA. In support of this contention was the correlation of LPS with sCD14. Brenchley et al first reported increased LPS levels in circulation during

chronic HIV infection and demonstrated that it was positively correlated with sCD14 [5]. Most studies in adults and in children [15, 20, 23] also concur with this finding showing correlation of LPS with sCD14. At week 44, the role of MT products in relation to IA was more definitive. T cell IA correlated with 16SrDNA and with sCD14, both of which correlated with each other, even though T cell IA did not correlate with LPS. Similar observations have been made in another recent pediatric study in which T cell IA did not correlate with plasma LPS [15]. The fact that even in VR patients, sCD14 was elevated at week 44 in the absence of overt viral replication, suggests that MT was involved in contributing to persistent elevation of this biomarker at a time when T cell activation decreased significantly. Finally, the finding that T cell activation in VF patients did not correlate with VL at week 44 but did correlate with 16SrDNA and sCD14 argues for a definitive role of gut MT in causing immune activation. In viremic individuals, immune activation and microbial translocation are associated with cycling of gut T cells suggesting that gut T cell activation and microbial translocation may be interconnected [30].

Elevated plasma sCD14 emerged as a consistent marker of ongoing pathology, and was not resolved with ART. Besides its function in LPS signaling, sCD14 might also play a role in inflammatory processes [31]. It is not known if the extent of monocyte/macrophage activation and resulting inflammatory conditions differ in perinatally infected children compared with infected adults who are known to manifest accelerated immune senescence with increased long-term morbidity and mortality from non-AIDS-related inflammatory conditions [32]. Interestingly, neonatal or cord blood-derived monocyte/macrophages manifest greater infectivity and HIV-1 replication [33]. A recent study of biomarkers in HIV-infected subjects in the SMART study showed that high plasma levels of sCD14 are associated with an increased risk of all-cause mortality [13]. In children as well, sCD14 has been reported to be the best single predictor of disease progression [15]. Altogether the prevailing data points to elevation of sCD14 as a more relevant biomarker of immune activation occurring as a consequence of MT.

One of the detrimental consequences of MT is in the context of IA and its association with impaired CD4 T cell reconstitution. Studies in adults have shown a negative correlation of MT products with CD4 T cell reconstitution. Although CD4 reconstitution was not a prominent feature of the patients in the study group represented here, there was an increase in CD4 counts in association with decrease in VL and T cell immune activation in the VR patient group. Plasma LPS was found to be negatively associated with baseline CD4% and absolute counts, and 16SrDNA was negatively associated with changes in CD4 following change in ART. Thus, together the data provide suggestive evidence for MT influencing CD4 reconstitution in this patient group.

We conclude that MT is a characteristic feature of HIV disease in the perinatally HIV-infected population. In the cohort reported herein, change of therapy from suboptimal ART to more potent treatment, which was still substandard for the current era, did not lead to decrease in MT even in patients deemed virologic successes. The MT products showed a strong relationship with monocyte activation which, in turn, correlated with T cell activation. Long term studies in children given early potent ART, as is the current standard of practice, could be informative for understanding gut healing and MT. The role of nutrition, antibiotic prophylaxis, and the impact of gut microbiota are also in need of investigation. Besides LPS, there are likely other sources of the microbial burden which could be responsible for chronic immune activation [11, 34]. Our data points to complementary information gained by concurrent study of LPS, 16SrDNA and sCD14 and suggests that inclusion of additional inflammatory biomarkers in longitudinal cohorts may help guide future strategies for maintaining gut integrity in HIV infected children.

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Figure 1. Plasma levels of microbial products and sCD14 (indicating monocyte activation) are elevated in HIV infected children; LPS does not correlate with 16SrDNA
 (A) Correlation between LPS and 16SrDNA; (B, C, D), plasma levels of LPS, 16SrDNA, and sCD14 respectively in total patients; (E), values of sCD14 in virologic responders (VR) and virologic failures (VF). Differences within groups were analyzed using Student's paired 2-tailed t-test.

Figure 2. Correlation of microbial translocation products with sCD14

(A), Correlation of LPS with sCD14 at baseline in total patients; (B, C), Correlation of 16S rDNA with sCD14 at week 0 and week 44 respectively in total patients; (D, E), Correlation of 16S rDNA with sCD14 at week 44 in VF and VR groups respectively. Spearman correlations (r and p values) were used to examine the relationships between the markers.

Figure 3. 16SrDNA correlates with T cell immune activation at week 44 in total patients and VF group, but not with VR group

16SrDNA copy number correlated with activated CD4 T cells; CD8 T cells respectively (A, B), in total patients; (C,D), Virologic failures; (E,F), Virologic responders. Spearman correlations (r and p values) were used to examine the relationships between 16SrDNA copy number and activation markers.

Figure 4. Monocyte activation (sCD14) correlates with T cell activation at Week 0 and Week 44 in total patients and in VF, but not in VR patients

Correlation of plasma sCD14 (A,B) with activated CD4 T cells at week 0 and 44 respectively in all patients; (C,D), with activated CD8 T cells at week 0 and 44 in all patients; (E,F), with activated CD4 T cells and activated CD8 T cells respectively at week 44 in VF; (G,H), with activated CD4 T cells and activated CD8 T cells respectively in VR at week 44. Spearman correlations (r and p values) were used to examine the relationships between sCD14 and immune activation markers (percentages of HLA-DR +CD38+) on CD4 and CD8 T cells.

Figure 5. T cell activation correlates with virus load at week 0 in total patients, not at week 44 in total patients or in VF

Virus load correlations with activated CD4 T cells and activated CD8 T cells respectively, (A, B), in total patients at week 0; (C, D), in total patients at week 44; and (E, F) in VF patients at week 44. Spearman correlations (r and p values) were used to examine the relationships between Log HIV RNA and immune activation markers (percentages of HLA-DR +CD38+) on CD4 and CD8 T cells.

Table 1

Characteristics of patient population

	Total			Viral Responders (VR)			Viral Failures (VF)		
	Week 0			Week 44			Week 0		
	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
HIV RNA (log₁₀ copy/ml)	2.6-5.7	4.23 (0.86)	2.6-5.8	3.39★ (0.86)	2.6-5.7	4.01 (0.97)	2.6-2.6	2.6★ (0.0)	2.6-6
% CD4	14-49.5	30.66 (7.92)	9-50	31.71 (8.67)	16-42.5	30.58 (7.46)	21-50	34.58‡ (6.98)	14-49.5
CD4 count	140-2991	854.3 (487.8)	51-2652	925.3 (492.4)	232-2940	935.1 (544)	248-2652	1027 (527.9)	140-2991
%CD4⁺DR⁺38⁺	2-78	12.34 (15.98)	0-60	9.41★ (13.12)	2-68	11.12 (13.16)	0-21	5.76★ (5.82)	2-78
%CD8⁺DR⁺38⁺	3-82	35.21 (17.34)	0-70	22.64★ (19.33)	3-76	33.72 (15.78)	0-59	16.61★ (16.93)	7-82

★ Significant decrease compared to Week-0;

‡ Significant increase compared to Week-0

Table 2

Correlation analysis of LPS, 16SrDNA and sCD14 with VL, CD4 and CD8

	Week 0			Week 44			Change	
	Correlation coefficient	p-value		Correlation coefficient	p-value		Correlation coefficient	p-value
LPS								
Log RNA	0.127	0.296		-0.018	0.882		-0.139	0.37
CD4 %	<u>-0.303</u>	<u>0.011</u>		0.097	0.475		0.294	0.09
CD8 %	0.139	0.249		0.161	0.236		-0.095	0.59
CD4 absol	<u>-0.357</u>	<u>0.002</u>		0.113	0.406		0.190	0.27
CD8 absol	-0.174	0.151		0.139	0.305		0.039	0.83
16S rDNA								
Log RNA	0.163	0.159		-0.028	0.811		0.114	0.35
CD4 %	0.032	0.782		0.032	0.810		-0.187	0.17
CD8 %	-0.102	0.380		-0.151	0.249		0.168	0.22
CD4 absol	-0.110	0.343		0.079	0.544		<u>-0.274</u>	<u>0.04</u>
CD8 absol	-0.125	0.281		0.033	0.802		-0.164	0.23
sCD14								
Log RNA	<u>0.248</u>	<u>0.022</u>		-0.042	0.703		-0.093	0.40
CD4 %	-0.079	0.473		0.104	0.396		0.179	0.14
CD8 %	0.030	0.648		-0.210	0.083		-0.230	0.07
CD4 absol	-0.096	0.381		0.014	0.912		0.170	0.16
CD8 absol	-0.041	0.705		-0.105	0.392		0.059	0.63

Relation of LPS, 16S rDNA and sCD14 with viral load, percentages and absolute counts of CD4 and CD8 at Week 0, Week 44 and change from Week 0 to Week 44 was examined using spearman correlations (r and p values).