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Increased Microbial Translocation in ≤ 180 Days Old Perinatally Human Immunodeficiency Virus Positive Infants as Compared with Human Immunodeficiency Virus -Exposed/Uninfected Infants of Similar Age

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Abstract

Background—We investigated the effect of early versus deferred antiretroviral treatment (ART) on plasma concentration of lipopolysaccharide (LPS) and host LPS-binding molecules in HIV-infected infants up to 1 year of age.

Methods—We evaluated 54 perinatally HIV-infected and 22 HIV-exposed/uninfected infants (controls) at the first and second semester of life. All HIV-infected infants had a baseline CD4 $\geq 25\%$, participated in the CIPRA-SA Children with HIV Early Antiretroviral Therapy (CHER) trial in South Africa and were randomized in: Group 1 (n=20), ART deferred until CD4 $< 25\%$ or severe HIV disease, and Group 2 (n=34), ART initiation within 6-12 weeks of age. LPS, endotoxin-core antibodies (EndoCAB), soluble (s)CD14, and LPS-binding protein (LBP) were measured in cryopreserved plasma. T cell activation was measured in fresh whole blood.

Results—At the first semester, LPS concentration was higher in HIV-infected infants than in controls; sCD14, LBP and T cell activation were higher in Group 1 than in Group 2 and controls. While LPS was not correlated with study variables, viral load was positively associated with sCD14, LBP or EndoCAB. At the second semester, LPS was not detectable and elevated host LPS-

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control molecules values were sustained, in all groups and in conjunction with ART in all HIV-infected infants.

Conclusions—While plasma concentration of LPS is higher in perinatally HIV-infected infants 0-6 months of age than in controls independent of ART initiation strategy, LPS-control molecules concentration is higher in infants with deferred ART, suggesting the presence of increased microbial translocation in HIV-infected infants with sustained early viral replication.

Keywords

HIV-1-infected infants; LPS; microbial translocation; LPS-binding molecules

INTRODUCTION

HIV-infection has been associated with increased permeability of the mucosal barrier resulting to translocation of microbes and/or microbial products into circulation without overt bacteremia (microbial translocation).¹⁻⁴ The role of microbial translocation, as a potential inflammatory stimulus resulting to HIV-associated pathogenesis acceleration, remains controversial.⁴⁻¹⁰ Lipopolysaccharide (LPS) a major component of Gram-negative bacterial cell wall and a potent immunostimulatory product,¹¹ which in several conditions^{8,12} reflects the degree of microbial translocation, has been shown by several studies to be increased in the plasma of HIV-infected subjects.^{4-6,13-15} Plasma LPS concentration is modulated by several host molecules, such as LPS-binding protein (LBP),¹⁶ soluble CD14 (sCD14),¹⁷⁻¹⁹ and naturally occurring immunoglobulin-M (IgM), IgA and IgG antibodies to the LPS core oligosaccharide (endotoxin-core antibodies, EndoCAb),^{20,21} that bind and/or neutralize circulating LPS. Introduction of antiretroviral therapy (ART) decreases plasma LPS concentration.⁶ ART interruption-associated viremia increases T cell activation acutely, yet LPS concentration changes are dependent on the viremic episode duration and the degree of LPS clearance by its ligands over time.¹⁵

Perinatal HIV infection is associated with a more rapid disease progression^{22,23} than in adults, yet little is known on plasma LPS concentration in HIV-infected infants, or on the effect of ART initiation strategy on microbial translocation or host LPS-control mechanisms. We report higher plasma LPS concentration in HIV-infected infants than in HIV-exposed uninfected infants, irrespective of ART initiation strategy, together with increased anti-LPS host responses and T cell activation.

MATERIALS AND METHODS

Participants

We studied 20 perinatally HIV-infected infants with deferred ART until CD4<25% or severe HIV disease (Group 1), 34 perinatally HIV-infected infants with ART initiated within 6-12 weeks of age (Group 2) and 22 HIV-negative infants born of HIV-infected mothers (controls). All HIV-infected infants had baseline CD4⁺ T cells $\geq$ 25%, and were enrolled in the CIPRA-SA Children with HIV Early Antiretroviral Therapy (ART) trial in South Africa (CHER).²⁴ Study variables were assessed sequentially in HIV-infected infants by $\leq$ 180 and >180-365 days of age (designated respectively as first and second semester of life), and compared against controls of similar age. All HIV-infected infants were ART-treated by the second semester of life. The study was approved by the Institutional Review Boards of the authors' institutions. Informed consent was obtained according to the Human Experimentation Guidelines of the US Department of Health and Human Services and the Authors' institutions.

LPS, Host LPS-Control Molecules and T Cell Activation Assessment

Whole blood was collected without prior fasting of the participating infants and used for plasma isolation and for assessment of CD8⁺ T cell activation. LPS concentration was determined by the Limulus Amebocyte assay according to the manufacturer's protocol (Cambrex Bioscience, Walkersville, MD) in plasma samples diluted 1/100 with endotoxin-free water and heated to 70°C for 10 min to inactivate plasma proteins. Plasma concentration of sCD14 (R&D, Minneapolis, MN), LBP (Cell Sciences, Canton, MA), and IgM EndoCAb (Cell Sciences) was determined in duplicate by enzyme-linked immunosorbent assay per manufacturer's specifications. Lower limits for LPS, sCD14, LBP, and EndoCAb were 0.1 EU/ml, 250 pg/ml, 781.25 pg/ml, and 0.055 MMU/ml, respectively. CD8⁺ T cell activation [percentage (%) of CD8⁺ T cells that are CD38⁺, HLA-DR⁺ and CD95⁺] was assessed by same day whole blood flow cytometry as previously described.²⁵

Statistical Analysis

Data analysis was restricted to subjects with CD4% and LPS measurements in either semester of life. Table 1 shows study variables statistics. Data are presented as medians with 25th-75th interquartile range (IQR) in parenthesis. Primary analysis aimed to assess differences across study Groups during the first semester of life for each of the 9 variables listed in Table 1 [with the exclusion of plasma HIV-1 RNA (log₁₀vl)]. In each case, a Kruskal-Wallis (K-W) test was performed to test for equality of medians across the Groups, with a multiple testing adjustment based on the approach of Benjamini and Yekutieli. Adjusted p-values with values that were less than a false discovery rate (FDR) threshold of 0.10 were considered statistically significant. Post-hoc pairwise comparisons between Groups for variables with a significant difference in medians across Groups, were performed using the Wilcoxon rank sum test.

The effects of Group assignment and visit age on LPS and LPS-control molecules were further characterized by fitting separate linear mixed effects models for each outcome. Random individual-level intercept terms, as well as fixed effects for visit age, Group and age by Group interactions were included in the model with the control Group as referent. Although analysis of study variables included both semesters of life, analysis of LPS was only limited to the first semester due to the high number of zero values in the second semester; in this case, each child had one observation, and so the random intercept was not included and the model reduced to the usual multivariable model. Monotonic transformations were applied in the case of non-normally distributed outcomes to ensure model assumptions were appropriate.

Pairwise correlations between (i) LPS and all other variables, (ii) the rate of LPS change with the rate of change of all other variables between the two visits, (iii) CD4% and all other variables, and (iv) log₁₀vl and all variables, were estimated using Spearman's correlation coefficient. Tests of the null hypothesis that the correlation was equal to zero were reported with unadjusted p-values. All statistical analysis was performed using R (vs.2.10.1).

RESULTS

Statistically significant differences across Groups for all primary study variables during the first semester of life

Table 2 shows the results of the Kruskal-Wallis (K-W) test performed to test for equality of medians across Groups for all 9 primary variables during the first semester of life, as well as the results of the multiple testing adjustment based on the approach of Benjamini and Yekutieli. As shown in Table 2, all 9 primary variables including LPS were statistically significant (FDR<0.10).

Increased LPS Concentration Associates with HIV Infection in the First Semester of Life

Post-hoc pairwise comparisons between Groups showed higher LPS concentration in both HIV-infected Groups, irrespective of ART initiation strategy ($p=0.03$ and $p=0.01$ for Groups 1 and 2 respectively) when compared with controls of similar age (see Figure, Supplemental Digital Content 1a). While the observed median of LPS concentration was slightly higher in Group 1 than in Group 2 (see Figure, Supplemental Digital Content 1a), the difference was not statistically significant ($p=0.56$).

The multivariable model confirmed that LPS was significantly greater in the HIV-infected Groups ($p=0.0073$ and $p=0.0174$ for Groups 1 and 2, respectively) than in the control Group (see Figure, Supplemental Digital Content 1b). Furthermore, this model revealed a marginal effect of visit age ($p=0.10$), though interactions between Group assignment and age on LPS concentration were not significant. Interestingly, LPS was not detectable in any child in the second semester of life (Table 1).

ART Delay Associates with Increased T Cell Activation and LPS-Control Molecules Concentration

In the first semester of life host LPS-control molecules concentration (sCD14, LBP and EndoCAb) and T cell activation levels were significantly higher in Group 1 than in controls or Group 2 (see Figure, Supplemental Digital Content 1a), consistent with a greater anti-LPS host response with deferred ART. In contrast to LPS, host LPS-control molecules were sustained throughout the first year of age (Table 1).

A MEM-based assessment over the first year (see Figure, Supplemental Digital Content 1b) suggested significantly greater values of sCD14 ($p=0.0496$) and LBP ($p=0.0045$) for Group 1 than in the control Group. This difference was not detected in the comparison of Group 2 to control. In all models, visit age led to estimated increases in the outcomes, although this was only statistically significantly different than 0 for sCD14 ($p=0.0228$).

Lack of association between LPS and host LPS-control molecules, T cell activation or plasma HIV-1 RNA during the first semester of life

There was no significant association detected between LPS and host LPS-control molecules or T cell activation in the first semester of life. Furthermore, no significant association was found between the rate of T cell activation or of host LPS-control molecules change between the two visits and the rate of LPS change [rate of change = (variable level at visit 2 - variable level at visit 1) / (visit age in days at visit 2 - visit age in days at visit 1)] (data not shown). Log₁₀VL was positively associated with CD8⁺ T cell activation (ex., % of HLA-DR⁺ CD8⁺ T cells, $p=0.002$, $\rho=0.7967$) and with host LPS-control molecules (see Figure, Supplemental Digital Content 1c), but not with LPS ($p=0.19$, $\rho=0.3238$).

DISCUSSION

To our knowledge, this is the first study investigating the potential role of ART initiation strategy on circulating LPS, host LPS-control molecules and T cell activation in HIV-infected infants up to 1 year of age.

In agreement with previous reports from us and others,^{15,26} we found in the first semester of life higher plasma LPS concentration in HIV-infected infants compared with controls of similar age. In contrast to our previous report showing higher concentration of LPS in ART naïve adults compared with ART-suppressed subjects,¹⁵ LPS concentration in both HIV-infected Groups in the first semester of life was not statistically different. This could be explained by the presence of viremia in both Groups independent of ART and by the fact

that due to the rapid deterioration of CD4% in Group 1, many children assigned to this Group were already undergoing treatment at the time of first sampling.²⁴

The detection of LPS in all infants in the first semester, independent of HIV infection status, suggests a basal level of bacterial translocation, which is higher in HIV-infected. This basal level of bacterial translocation into circulation could be attributed either to increased vulnerability of the immature immune system,²⁷ or, since all infants were formula fed and did not fast before sample's collection, to increased permeability of the gut due to high fat diet,²⁸⁻³⁰ presence of LPS in the formula,³¹ or presence of LPS in the water used for formula preparation. Overall though, the above factors, although explaining the basal translocation observed in all groups, cannot account for the increased levels observed in HIV-infected, as infants in all groups were age-matched and part of the same nutritional program, further supporting the association between HIV infection and increased microbial translocation.

The detected LPS concentration was similar to that reported in adults¹⁵ yet higher than that reported by Wallet et al in older HIV-infected children.²⁶ Our findings suggest a comparable impact of HIV infection on plasma LPS concentration between HIV-infected infants and adults despite different disease progression outcomes. However, the presence of viremia and increased LPS concentration in HIV-infected infants during the first semester of life may have a greater significance for health as the CHER study showed increased death rate and HIV progression in Group 1 compared with Group 2, a finding that triggered the initiation of ART in all infants initially randomized in Group 1 and influenced treatment guidelines worldwide (i.e. ART is initiated as soon as possible after HIV diagnosis regardless of CD4% levels or disease progression).²⁴

T cell activation was higher in HIV-infected infants and in positive correlation to viral load confirming that early ART initiation is beneficial in reducing cellular activation, even if its effect on LPS concentration is not immediately evident. Exposure to other pathogens or nutrition-related factors such as bottle feeding, which was common to all infants, could also contribute to increased inflammation due to lack of breast milk IgAs³² or of other soluble maternal components that could mediate anti-inflammatory effects.^{26,33} Lack of association between T cell activation and LPS or between T cell activation rate of change and LPS rate of change, although in agreement with our previous findings in adults¹⁵ and the report from Wallet et al.,²⁶ stands in contrast to the study of Brenchley et al⁶, a difference which could be due, in part, to differences in study design.

Host LPS-control molecules were higher in HIV-infected infants with deferred ART compared with HIV-negative infants born of HIV-infected mothers and were correlated with viral load suggesting an early development of a defensive neutralizing response to LPS in conjunction with higher concentration of LPS. This is consistent with previous reports in adults showing increased EndoCAB IgM concentration in conditions of microbial translocation,^{21,34-36} and increased sCD14 concentration in HIV-infected adults.^{6,15,26,37,38} Furthermore, in light of recent data suggesting that sCD14 is an independent predictor of mortality in HIV infection,³⁹ our data showing in HIV-infected infants with deferred ART higher levels of sCD14, LPBP and EndoCAB in the first semester of life, together with significantly greater values of sCD14 and LBP over the first year, further support the need for longitudinal studies investigating whether these molecules could be more clinically relevant than LPS as they serve as measures of the host response to microbial translocation. We interpret the lack of direct association between plasma concentration of LPS and LPS-host molecules to reflect an insufficient clearance of LPS due to sustained microbial translocation above levels neutralized by host LPS-control molecules.^{26,40}

Lack of LPS detection in the second semester was independent of HIV infection and cannot be attributed to technical problems, as samples for both semesters were tested at the same time in a blinded fashion. This finding contrasts with the results of Wallet et al.²⁶ showing persistent microbial translocation following ART-mediated immune reconstitution, a difference which could be attributed to differences in patient cohorts. The lack of detectable LPS in the second semester in all groups suggests a possible role of other factors besides ART in decreasing LPS (i.e. cease of the formula program and transition to solid foods in all infants at 180 days, efficient LPS control by sustained levels of LPS-host molecules, or immune system maturation). The lack of second semester values for LPS in all subjects where first semester values are reported, could raise the potential for informative dropout. We interpret this not to be likely, as LPS measurements after 180 days were undetectable and no a priori reason was identified for missing values when present. Importantly, our study does not address long-term effects of sustained viremia after 180 days as ART was introduced in 84.3% of the HIV-1-infected infants.

In summary, in the first semester of life we show higher LPS concentration in all HIV-infected infants independently of ART initiation strategy as compared with controls of similar age, together with a decrease in LPS concentration to undetectable levels by one year of age in all infants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Statistics of study variables

Variable	N	control median(25th,75th IQR)	N	Group 1 median(25th,75th IQR)	N	Group 2 median(25th,75th IQR)
First semester of life						
Birth weight (gr)	22	2575(1900,3030)	20	3095(2585,3375)	34	3000(2700,3200)
CD4 %	22	42.85(40.1,45.5)	20	32.4(23.4,37.4)	34	38.3(29.2,42.3)
Viral load(log10 copies/ml)	0		9	5.9(5.1,5.9)	9	3.1(3.0,3.3)
LPS (EU/ml)	22	6.3(3.3,9.5)	20	15.9(5.1,28.3)	34	11.1(5.2,21.6)
sCD14 (pg/ml)	22	1292792(1139196,1597271)	20	1741948(1533036,1965577)	34	1208910(1096681,1583754)
LBP (pg/ml)	22	3858329(1545423,7029187)	20	10738199(4406019,17381400)	34	2964262(1624802,6939960)
EndoCAb (MMU/ml)	22	32.3(20.5,59.4)	20	70.9(28.8,183.9)	34	26.5(9.7,51.1)
CD38+ % of CD8+ T cell	19	98.3(97.7,99.5)	10	99.7(99.5,99.9)	22	97.7(97.0,99.0)
HLA-DR+ % of CD8+ T cell	19	3.9(1.9,9.9)	10	43.2(30.4,47.7)	22	8.3(4.0,16.4)
CD95+ % of CD8+ T cell	19	91.6(88.2,95.5)	10	97.0(89.3,99.5)	22	70.7(46.3,85.9)
Second semester of life						
Birth weight (gr)	7	3100(2960,3325)	13	2855(2420,3000)	27	2900(2700,3100)
CD4 %	8	39.85(33,46.1)	13	34.9(29.3,39.8)	27	40.1(34.5,42.5)
Viral load(log10 copies/ml)	0		4	5.9(5.7,5.9)	5	4.5(4.1,5.9)
LPS (EU/ml)	8	0(0,0)	13	0(0,0)	27	0(0,0)
sCD14 (pg/ml)	8	1775062(1137080,2416197)	13	1733605(1493068,2119054)	27	1771584(1247540,1907024)
LBP (pg/ml)	8	5804629(3214348,15961410)	13	8877058(5029656,23264046)	27	4284089(2383678,7301718)
EndoCAb (MMU/ml)	8	43.9(32.8,62.0)	13	54.5(32.5,62.7)	27	29.6(26.8,40.7)
CD38+ % of CD8+ T cell	3	97.8(96.9,98.5)	8	99.6(98.8,99.8)	16	98.8(96.5,99.7)
HLA-DR+ % of CD8+ T cell	3	41.6(41.3,47.3)	7	15.9(9.5,34.7)	16	23.6(7.0,39.6)
CD95+ % of CD8+ T cell	3	89.1(83.7,89.4)	7	93(85,96.9)	16	86.1(66.0,94.4)

TABLE 2

Primary analysis during the first semester of life

Variable	N (N _C , N _{G1} , N _{G2})*	Kruskall-Wallis test (Unadjusted p value)	Kruskall-Wallis test (Adjusted p value) [†]
Birth weight (gr)	76 (22, 20, 34)	0.02326	0.0703
CD4 %	76 (22, 20, 34)	0.00015	0.0019
LPS (EU/ml)	76 (22, 20, 34)	0.02486	0.0703
sCD14 (pg/ml)	76 (22, 20, 34)	0.00056	0.0028
LBP (pg/ml)	76 (22, 20, 34)	0.00083	0.0035
EndoCAb (MMU/ml)	76 (22, 20, 34)	0.02328	0.0703
CD38 ⁺ % of CD8 ⁺ T cell	51 (19, 10, 22)	0.00054	0.0028
HLA-DR ⁺ % of CD8 ⁺ T cell	51 (19, 10, 22)	0.00002	0.0005
CD95 ⁺ % of CD8 ⁺ T cell	51 (19, 10, 22)	0.00055	0.0028

* N: total number of subjects having the corresponding variable readings; N_C: number of subjects in control Group having the corresponding variable readings; N_{G1}: number of subjects in Group 1 having the corresponding variable readings; N_{G2}: number of subjects in Group 2 having the corresponding variable readings.

[†] Multiple testing adjustment based on Benjamini and Yekutieli approach.