882 MICROBIAL TRANSLOCATION, IMMUNE ACTIVATION, AND GUT DYSBIOSIS IN HIV-EXPOSED INFANTS (the MITABS study).

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Abstract:

Introduction: The **MITABS study (**Microbial Translocation[MT], Immune Activation[IA], and Altered Bowel Flora Study[ABS]) is the first prospective, longitudinal study designed to simultaneously assess MT, IA, and alterations in the gut microbiome with clinical events in young (enrolled at < 6 months) HIV perinatally infected (HEI); and perinatally exposed, uninfected(HEU) infants in the Dominican Republic (DR) before and after starting antiretroviral therapy(ART). **Methods:** Cellular immune markers of IA (HLADR+CD38+ coexpression) in CD4 and CD8 T cells were determined by flow cytometry using the method of Hanekom et al, J Immune Meth, 2004. Plasma MT and IA (LPS, sCD14 and sCD163) were determined by standard assays. Metagenomic analysis was performed on both stool and plasma. Simultaneous assessments of MT, IA, ABS, and clinical events occurred at Entry, 6 weeks, 3,6, 12, and 18 months post entry. No HEI was on ART at entry. HIV-exposed infants in the DR are formula fed. **Results:** Between 6/6/13 and 3/1/17, 78 infants (31 HEI; 47 HEU) were enrolled in the DR. Median ages at entry for all 78, the 31 HEI and the 47 HEU were 106, 145, and 89 days respectively. 19/31 HEI started ART. 10/31 developed AIDS (CDC criteria); 4 of whom died. No HEU has died. At entry, compared to HEU of the same age (<6 mo, n= 44), HEI not on ART (n= 15) had higher CD8 T cell Immune activation (HLADR+CD38+, 23.6% vs 4.3%, p=0.0005),CD8 (47% vs 24%, p=0.001), lower CD4 (49% vs 71%, p= 0.002) and lower CD4/CD8 ratio (1.04 vs 2.9). HEI also had higher plasma sCD14 (2523 vs 1473ng/ml, p=0.0001), and a trend for higher sCD163 (1212 vs 932 ng/ml, p=0.06) but surprisingly, the 2 groups had similarly elevated markers of MT (LPS, HEI 250 vs HEU 249pg/ml; iFABP, HIE 3293 vs HEU 2755 pg/ml). On prospective follow-up, MT markers (LPS, sCD163) in HEU normalized by 6-9 months. T cell IA were all within the normal range in HEU over time. In HEI, although IA and MT values decreased following ART, they were higher than in HEU. HEI gut microbiome was associated with lower diversity (richness, n=13) compared to HEU (n=38), and an unknown member of the Megaspheara genus was enriched in HEI on ART compared to HEI not on ART. **Conclusions:** HEU infants like HEI have high gut permeability during early infancy which gradually normalizes over time. Increased biomarkers of MT and IA in HEI are prevalent from early infancy and persist after starting ART. HEI have a less diverse microbiome than HEU, with enrichment of the genus Megaspheara in HEI on ART.

Background:

Chronic inflammation is the primary driver of HIV disease progression. Within the last dozen years, Microbial Translocation (MT) has been recognized as a major contributor to this chronic immune activation. MT occurs in SIV-infected macaques and both HIV-infected adults and children but most of the data collected to date has been retrospective in nature. The **MITABS (**Microbial Translocation[MT], Immune Activation[IA], and Altered Bowel Flora Study[ABS]) study was designed as a prospective, longitudinal study designed to assess MT, IA, and alterations in the gut microbiome with clinical events among HIV-perinatally-exposed Dominican infants and children in Santo Domingo, the Dominican Republic. The Specific Aims were as follows: Specific Aim#1. Determine whether the persistently increased cell-surface markers(CD8+CD38+, CD8+DR+, and CD8+CD38+DR+ T cell subpopulations) of immune activation on CD8+ lymphocytes correlate on longitudinal testing with increased plasma levels of biomarkers of microbial translocation (Lipopolysaccharide[LPS], sCD14, sCD163, and iFABP) among prospectively followed, young, newly diagnosed HIV perinatally infected infants when compared with perinatally exposed, uninfected infants. Specific Aim#2. Determine whether perinatal HIV-1 infection is associated with a consistent shift in the microbial community diversity and composition of the gut microbiome (enteric gut bacterial flora genome) of perinatally infected children in various stages of their HIV disease relative to perinatally exposed but uninfected children.

Methods:

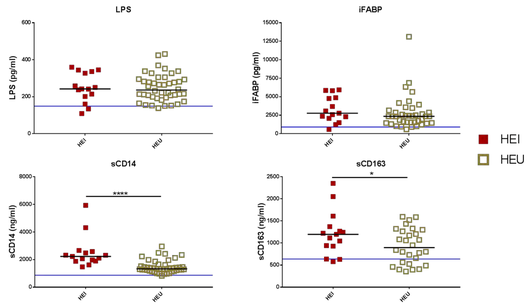
Prospective Observational Study that simultaneously assessed clinical, immune activation, microbial translocation, and alterations in the intestinal microbiome in young (enrolled at < 6 months) HIV perinatally infected (HEI); and perinatally exposed, uninfected(HEU) infants in the Dominican Republic (DR) before and after starting antiretroviral therapy(ART). Infants were enrolled by 6 months of age (Baseline/entry visit; pre-ART), and seen at 6 weeks, 3, 6, 12, and 18 months post entry. Clinical assessment, IA by FACS, MT(LPS, sCD14, 16SrDNA, sCD163), and Metagenomic Assessment of stool at each time point. Cellular immune markers of IA (HLADR+CD38+ coexpression) in CD4 and CD8 T cells were determined by flow cytometry using the method of Hanekom et al, J Immune Meth, 2004. Plasma MT and IA were determined by standard assays. Metagenomic analysis was performed on both stool and plasma. Simultaneous assessments of MT, IA, ABS, and clinical events occurred at each visit. Data analysis was performed by both visit number and age grouping. No HEI was on ART at entry. HIV-exposed infants in the DR are provided formula for the first 6 months of life. 16S analyses from stool samples were performing using the QIIME and R software packages.

Results:

Between June 6, 2013 and March 1, 2017, 78 infants HIV-perinatally exposed infants were enrolled in the DR; 30 were perinatally infected (HEI); while 48 were perinatally exposed and uninfected (HEU). The mean and median age at entry for 30 HEI was 181+/-103 and 145 days while the mean and median age for the 48 HEU was 109+/-33 and 89 days respectively. There was no difference in gender between the Cases and the Controls (HEI: 56.7% female; HEU: 58.3% female).
Eighty-eighty-five % of the Cases /and Controls were WH (Dominican) while the rest were Black (Haitian).

Results:

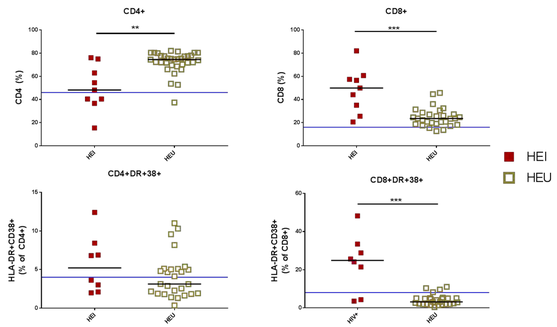
Soluble markers of Immune Activation and Microbial Translocation at baseline in HEI and HEU



Compared to HEU, HEI showed higher concentration of markers of monocyte activation (sCD14 and sCD163) but no differences in the markers of microbial translocation. Moreover, all the soluble markers evaluated are higher in both HEI and HEU compared to the level registered in healthy children of similar age.

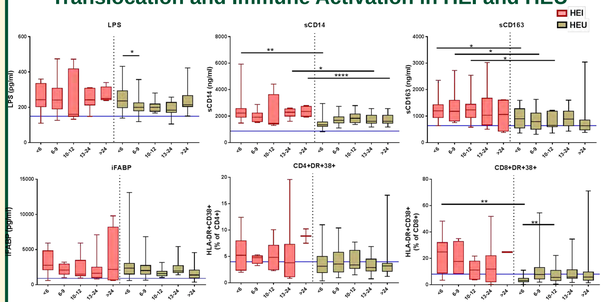
Results:

T cell Immune Activation at baseline in HEI and HEU



HEI also showed a strong perturbation of the T cells compartment with lower frequency of CD4+ T cells and higher frequency of CD8+ of which a higher proportion is immune activated (HLA-DR+CD38+)

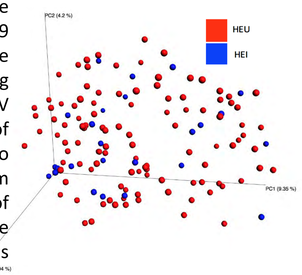
Longitudinal evaluation of the markers of Microbial Translocation and Immune Activation in HEI and HEU



The T cell immune activation is restored with ART initiation while the soluble markers of macrophages activation persist

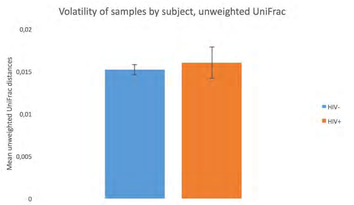
Beta Diversity Analyses of HEI and HEU subjects

Beta diversity tests were performed using 21839 sequences per sample. These showed limited clustering using unweighted UniFrac by HIV status. Adonis tests of clustering also showed no significant patterns, apart from a weak but significant effect of the subject's hospital. An age gradient, as expected, was observed.



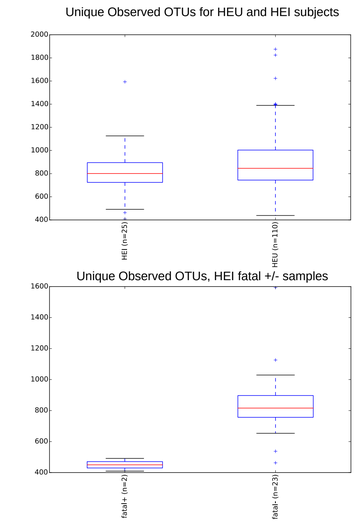
Microbiome Volatility Analyses

We observed a higher volatility (beta-diversity distances between time points) for HEI subjects which can be an indicator for microbial dysbiosis, however, this was not significant.



Alpha Diversity Analyses

The overall diversity within a community, alpha diversity, was measured. Multiple metrics, including observed OTUs, shannon evenness, and Faith's phylogenetic diversity showed a trend of higher diversity for HEU subjects. A linear mixed-model was used to account for subject non-independence, but yielded non-significant differences between HEI and HEU subjects. Within HEI samples, two subjects died and these showed much lower diversity than other HEI samples.



Within HEI subjects, HAART-compliance was linked significantly to an enrichment of one *Megaspherea* spp. OTU. This was calculated using a linear-mixed model with subject as a random effect.

Conclusions

1. HEU infants like HEI have high gut permeability during early infancy which gradually normalizes over time.
2. Increased biomarkers of MT and IA in HEI are prevalent from early infancy and persist after starting ART.
3. HEI have a less diverse microbiome than HEU, with enrichment of the genus Megaspheara in HEI on ART.

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