PROFILING OF GENES INVOLVED IN INFANT IMMUNE RESPONSES: A Transcriptional Comparison Between HIV-Exposed Uninfected And HIV-Unexposed Uninfected Infants in Kilifi, Kenya.

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Introduction

Vertical transmission of HIV-1 has reduced over the years due to the introduction of prevention mechanisms such as HAART, obstetrics management and reduced breastfeeding. This has led to a decrease in the HIV infected infant population giving rise to a new population termed as the HIV exposed uninfected infants (HEU); infants born to HIV positive mothers but are themselves not infected.

The HEU infant has been found to have an increased hospitalization and mortality rate when compared to the HIV-unexposed uninfected (HUU) infants; infants born to HIV negative mothers. Whether this is as a result of environmental exposure or intrinsic immunological alterations is not clear. This study focuses on the effect of the exposure to HIV antigens and the altered *in utero* cytokine milieu to the immune system of the HEU infant that may have led to the immunological alterations.

Objectives

The overall objective of this study is to use a transcriptomic approach to determine whether expression profiles of genes relevant for immune responses differ between HEU and HUU infants.

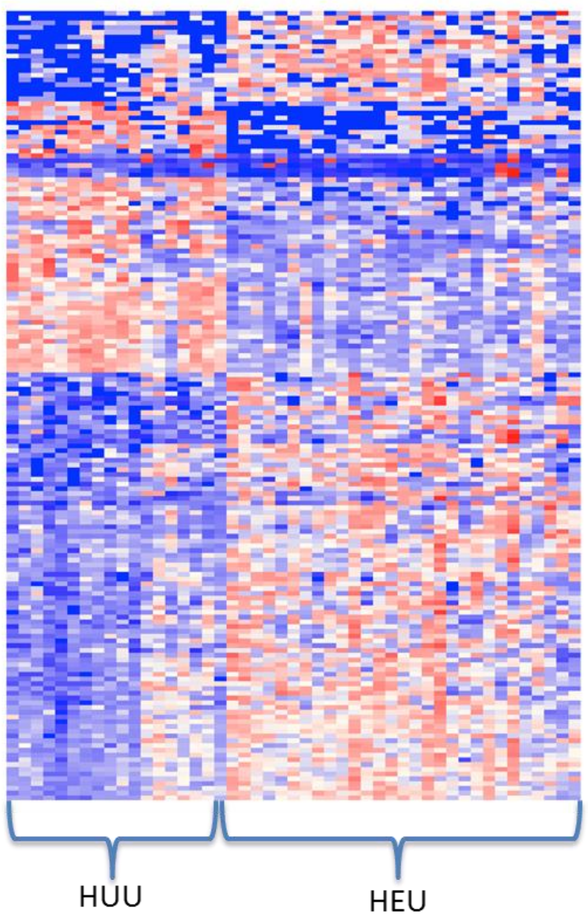
- To design an RNA-Seq analysis pipeline.
- To identify differentially expressed genes in age-matched HEU and HUU infants in the first two years of life.
- To compare gene expression profiles between HEU and HUU infants for genes relevant to the developing immune system.

Methods

- Quality of the raw reads was checked using FastQC software.
- Fast files were aligned to the splice-aware Tophat2 software using the human reference and GTF file,GRCh38vs86.
- The estimated abundance levels were quantified at exon level using HTSeq.
- The quantified abundance estimates were combined into a DESeqDataSet object and normalization done on them using the geometric mean method incorporated in the DESeq2 package.
- Differential gene expression of the samples was analysed using a negative binomial distribution model as per the DESeq2 package.

Results

(1a)



(1b)

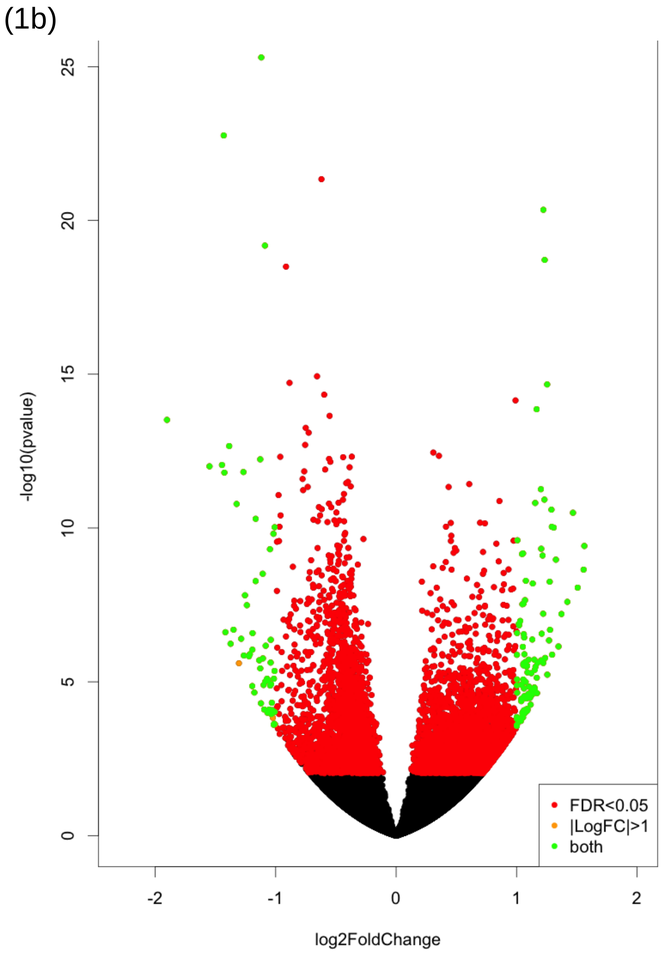


Fig 1. Differential expression (a) Heatmap for the differentially expressed genes in HEU at FDR <0.05, 5,416 genes in total. Red indicates upregulated genes while blue indicates downregulated genes. (b) Volcano plot for the significantly differentially expressed indicated by the green dots at FDR < 0.05 and Log2FoldChange >|1|.

(2)

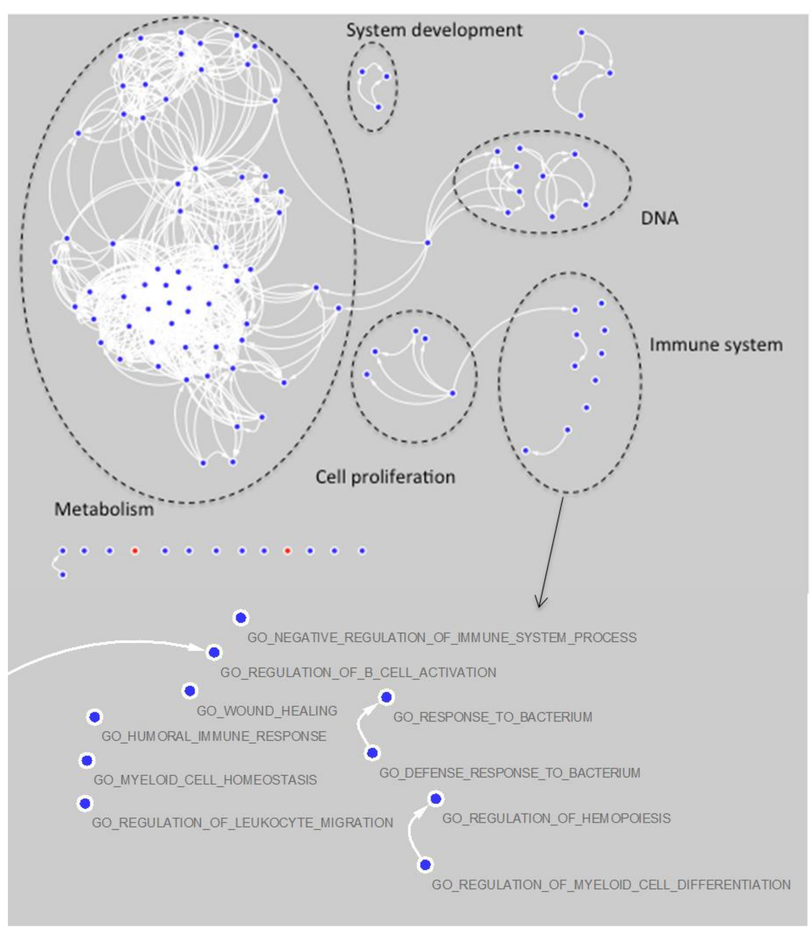


Fig 2. Gene sets associated with the immune system. The gene ontologies are formed by grouping genes that perform the same function into gene sets. The gene sets are represented by the nodes while the edges represent the GO defined relations. The blue nodes represent downregulated gene sets while the red nodes represent the upregulated gene sets.

(3)

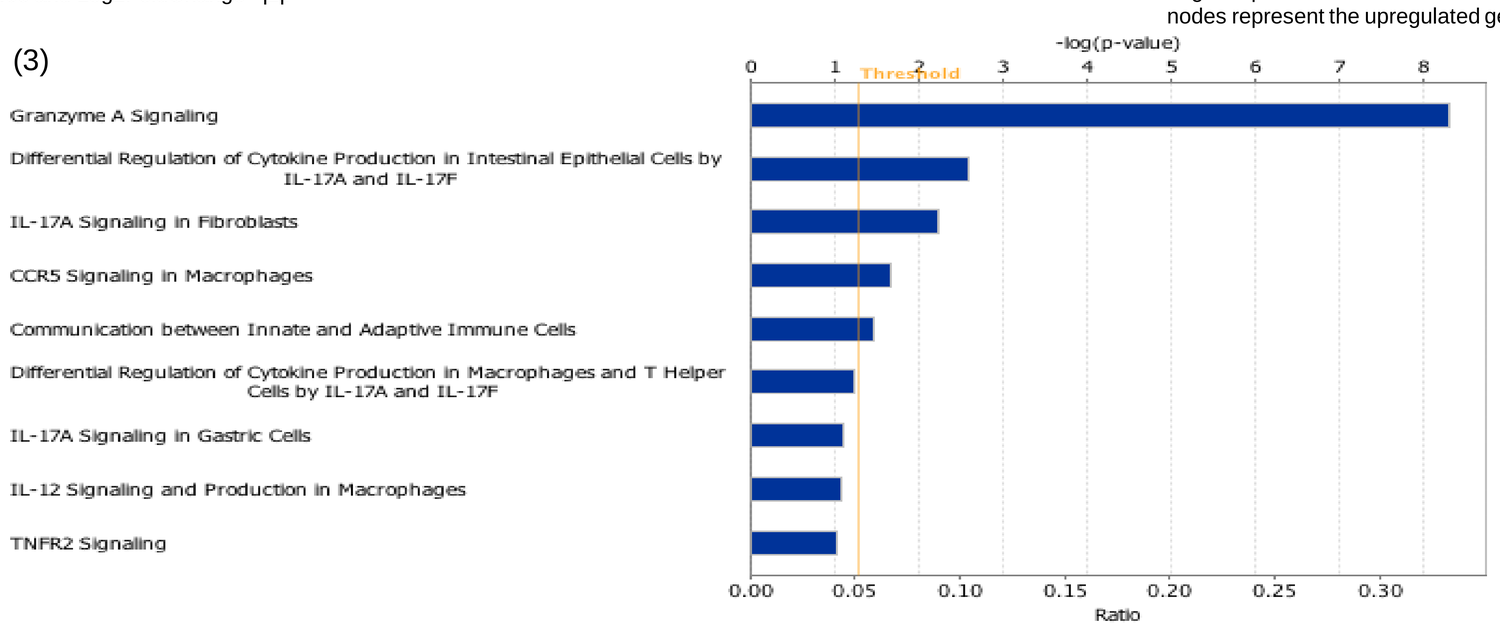


Fig 3. HIV exposure alters the Granzyme A pathway significantly. Canonical pathways associated with the 166 statistically significant differentially expressed genes were determined and a p value < 0.05 was used as a threshold cut-off. Granzyme A signalling pathway was significantly altered with a p value of 4.79E-09.

Conclusions and Future work

- Exposure to HIV antigens, despite the absence of infection, does contribute to distorting the HEU's immune system.
- Most of the genes associated with neutrophils irregularly expressed probably leading to a compromised innate immune system.

- Analysis with a non-restricted subset of samples and further analysis to whether ARV exposure and breastfeeding are a contributing factor.
- Future work looking at a later time point once this children are not on active clinical follow up because the HEU infants were on Cotrimoxazole.
- Confirmation of the expression of the CISH, FFAR2, HCAR3, CAMP, HMGB2, HP and LCN2 genes in the HEU infants through PCR.

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