Lasting Immune Impacts of Age at Start of ART in Vertically HIV-Infected Adolescents

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Background

Timely implementation of antiretroviral therapy (ART) in vertically HIV-infected children provides an early opportunity to restrict the establishment of infection and limit the size of reservoir. What is not fully understood is how early ART must begin, nor whether there is an effect on host immune via delayed intervention. With the hypothesis that later ART treatment can durably impact host immune responses that associate with HIV infection, we analyzed HIV-specific CD4 T cell functionality along with RNA-Seq transcriptional assays and pathway-based bioinformatics to identify a candidate gene expression signature in young HIV-infected adults that can distinguish between their early or later start of ART after birth.

Methods

PBMCs were collected from young HIV-infected adults between 9-15 years of age enrolled at Bambino Gesù Children’s Hospital. Three groups of donors (n=5-f/group) were profiled via RNA-Seq: (i) Early Treated (ET); Age at ART 0-5 Y, (ii) Late Treated (LT): Age at ART 1-12 Y, and (iii) HIV negative controls. Duration of ART at time of first sample (T0) in this study was approximately 3 to 15 years. All HIV-infected donors had been durably suppressed for plasma HIV. Low input libraries were generated using Roche/Kapa mRNA HyperPrep Kits and sequenced on an Illumina NextSeq 500 (75 base pair, paired-end, 40 million reads/sample). Differentially expressed genes (DEG) were determined by two-group t tests (P≤0.05) and organized into top pathways by P value (P≤0.05) via gene set variation analysis (GSVA) in R Biocductor, with pathway annotation from mSigDB [https://software.broadinstitute.org/gsea/msigdb] or Ingenuity Pathway Analysis. Antigen-specific CD40L+ CD4 T cells were evaluated by flow cytometry for intracellular cytokines (IL2, IFNG, TNFA, IL21) following 18hrs stimulation with gp140.

Results

Start with a simple RNA-Seq contrast : Early vs Late Start of ART

Figure 1. Pathway analysis of 1,328 differentially expressed genes by contrast of early vs late start of ART in adolescents who were vertically infected with HIV. Top enriched pathways (source: mSigDB Immunologic Signatures Database) by P value are shown vertically (P≤0.05) along with their populating genes (all P≤0.05, top row) and gene expression on the horizontal (Red = upregulated at earlier start of ART vs late, blue = downregulated). Note that the candidate T cell gene expression signature that distinguishes early vs later start of ART in PBMCs at adolescence can therefore also be found in immunophenotyping studies published at the Gene Expression Omnibus involving naive vs effector and central memory CD4 T cell signaling.

Regression Analysis with Immuno-Phenotyping Data

Figure 4. With regression analysis between DEG and CD4+CD27+CD45RO+ and CD4+CD27-CD45RO- expression by immuno-phenotyping at T0, a signature emerges that correlates or anti-correlates with effector or naive CD4+ T cells, respectively. However, age still largely correlates or anti-correlates with this signature.

How do we to separate the impact of age from the impact of time of start of ART?

Figure 3: Filtering out hundreds of genes associated with healthy aging in children (4-9 Y) versus young adults (10 – 20 Y) reveals a more relevant gene expression signature. We removed baseline gene expression from another pediatric study that distinguishes pre- and post-pubertal children in a Miami influenza vaccination dataset (IMpACT). We uncovered a closer age related signature (left) that includes CTSS known to increase over time in HIV infection. ELT genes that have been associated with effector T cell memory responses in SIV challenge vs challenge studies, and similarity with other CD4 memory development datasets in infectious disease (pathway analysis below).

Assessing HIV-Specific CD4 T Cell Functionality

Figure 6: We analyzed HIV-specific T cell functionality by stimulating CD4 T cells from the HIV-positive individuals in this study for 18hrs with gp140. Intracellular cytokines were evaluated by flow cytometry (ICS).

CONCLUSIONS

• Our results suggest that delayed ART has a lasting impact on host T cell memory distinguishable by a candidate DEG signature and HIV-specific immune responses.

• Regression analysis revealed that different patterns of T cell signaling pathway DEG correlated with age, age of ART initiation, and % of naive and effector memory T cells, e.g. chemokines/chemokine receptors, stress and effector molecules, and TNF superfamily signaling.

• Through pediatric age-related gene expression filtering and pathway analysis, a candidate signature of impact of start of ART was ascribed to differences mainly seen in naive vs central and effector CD4 memory T cell datasets.

• The frequency of gp140+CD4+ T cells was the same between ET and LT, but ET showed increased frequencies of gp140-specific polyfunctional.

• Expanded studies that control for age across treatment groups at time of sampling are warranted to assess these profiles of host immunity in vertically HIV-infected children treated with ART after birth and whether they can be targeted in functional cure approaches.

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