CHARACTERIZATION OF THE INFLAMMATORY CYTOKINE PROFILE AND LATENT HIV RESERVOIR IN EARLY TREATED AND LONG-TERM SUPPRESSED HIV INFECTED CHILDREN – THE EMERGING INSIGHTS INTO HIV PERSISTENCE

Shalena Naidoo[1]; Michele D. Sobolewski[2]; Taylor Simmons[2]; Joshua C. Cyktor[2]; Melissa A. Tosiano[2]; Mary-Grace Katusiime[1]; Mark F. Cotton[3]; Gert U. Van Zyl[4]; John W. Mellors[2]; Richard H. Glashoff[5]

[1]Stellenbosch University, Division of Medical Virology, Cape Town, South Africa;
[2]University of Pittsburgh, Pittsburgh, Pennsylvania, United States of America;
[3]Stellenbosch University and Tygerberg Children's Hospital, Cape Town, South Africa;
[4]Stellenbosch University, Division of Medical Virology and National Health Laboratory Services (NHLS), Cape Town, South Africa;
[5]Stellenbosch University, Immunology Unit, Division of Medical Microbiology and National Health Laboratory Services (NHLS)

Background

Knowledge of immunological mechanisms contributing to HIV persistence in early treated children is limited. It is unknown whether the few HIV infected cells that persist on long-term ART harbor proviruses capable of infectious virion production. This study compared inflammatory cytokine levels in virologically suppressed children soon after therapy initiation and at 7-8 years. The frequency of inducible HIV from CD4+ T lymphocytes were also determined.

Methods

Twenty-six cytokines were measured by multiplex-assays in plasma samples at baseline (6 months) and 7-8 years. Samples originated from the CHER trial and included three study arms (Arm 1: delayed therapy; Arms 2 and 3: early therapy until interruption at 40 and 96 weeks). Children began ART at <1 year of age and sustained viral suppression at 7-8 years. A subset of participants was also tested for total HIV-1 DNA using qPCR targeting a conserved region in HIV *integrase.* Total inducible virus recovery (TVR) assays were performed on purified CD4+ T cells stimulated with PMA and ionomycin. Virus outgrowth assays (VOA) were also performed on purified CD4+ T cells, activated with phytohemagglutinin and co-cultured with irradiated HIV-1 negative feeder cells and CD8-depleted blasts. HIV-1 RNA was measured in culture supernatants using a quantitative HIV-1 PCR assay targeting *integrase* (iSCA) with single-copy sensitivity. VOA supernatants were assayed for p24 antigen by EIA.

Results

Thirty-eight samples were evaluated for cytokine expression. The median baseline viral load at ART initiation was 738,500.5 copies/ml (range: 399–750001 copies/ml). The median CD4 percentage at baseline and follow-up were 36.9% (range: 23.1-57.1%) and 37.5% (range: 27-47%) respectively. In Arm 1, significantly higher levels of INF-γ ($P = 0.0117$), IL-17A, TNF-α, RANTES (all $P = 0.0039$) and G-CSF ($P = 0.0078$) were observed at baseline. A significant increase in cytokine expression was observed for IL-13, IL-4 ($P = 0.0156$), VEGF, MCP-1, and PDGF-BB (all $P = 0.0039$) at follow-up. Arm 2 showed highly significant elevations at follow-up for IL-13 ($P = 0.0005$), VEGF ($P < 0.0001$), FGF-basic ($P < 0.0001$), MCP-1 ($P < 0.001$), and PDGF-BB ($P < 0.0001$). Similar trends were noted for Arm 3. In 23 children assessed for HIV-1 DNA at follow-up, a median of 32.5 copies/million cells (range: 0–247.6) was observed.

TVR and VOA were implemented on 10 PBMC (6 females, 4 males) at 7-8 years of age. Five participants had pre-ART HIV RNA >750,000 copies/ml and 5 had a median pre-ART HIV RNA of 635,000 copies/ml. The median CD4 percentage at 7-8 years was 39%. Median cell associated HIV-1 DNA was 38 copies per million cells (range: 4.5-186). Two of 10 children had inducible HIV-1 RNA by TVR assay detected by iSCA at 2267 and 24 copies/ml. All VOA supernatants collected were negative for p24 antigen, but 4 participants had detectable HIV-1 RNA (range: 9-697 copies/ml).

Conclusion

Despite the low level of cell-associated DNA detected, children initiated on ART early showed an increase in chronic inflammatory disease-associated cytokine expression following 7-8 years on suppressive therapy. Although infectious virus could not be readily isolated in viral outgrowth assays, inducible virion production was detected in 4 of 10 children using a highly sensitive HIV-1 RNA assay.