Project: Biomarkers of Inflammation and Endothelial Activation in Vertically HIV-1 Infected Children

Applicant: Jeremy Soo, University of Alberta School of Medicine

Supervisor: Dr. Michael Hawkes, Department of Pediatrics, University of Alberta

Background

Mother-to-child transmission (MTCT) is the primary method of pediatric human immunodeficiency virus 1 (HIV-1) infection. Presently, ~3.2 million children are living with HIV-1 infection and approximately 240 000 infants are newly infected every year [1]. Although there is currently no cure for HIV, the use of combination antiretroviral therapy (cART) has significantly increased the life expectancy of infected individuals and decreased transmission of the virus. However, long term complications are emerging even after successful chronic cART. Higher levels of inflammation and endothelial activation still persist in HIV infected individuals despite fully suppressed viral replication [2]. These factors are thought to increase risk of cardiovascular disease, yet their contribution in HIV infection remains unclear [3,4].

A recent study among HIV infected infants found that immune activation occurred early in perinatally-infected infants, with higher levels of soluble CD14 (sCD14), interleukin 6(IL-6) and C-reactive protein (CRP) by 6 weeks of age compared to HIV-exposed uninfected or HIV- unexposed infants [5]. Elevated levels of proinflammatory cytokines (Tumor necrosis factor (TNF-α), IL-6, IL-1β,)) have been implicated in loss of lean body mass, growth impairment, and poorly controlled HIV viral replication in children [6-8]. Other inflammatory biomarkers that have been documented in its association with chronic HIV-1. Some examples include the CXC-cytokine 10 kDa interferon gamma-induced protein (IP-10) and the soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) [9-12]. However, IP-10 and sTREM-1 have only been documented in adults and have not yet been investigated in infants with HIV-1.

There has also been increasing evidence to suggest that endothelial activation plays a key role in pathogenesis and disease progression of HIV-1. A pro-inflammatory state and endothelial activation persist in patients with chronic, well-controlled HIV-1 infection for as long as 12 years after successful antiretroviral therapy[2]. Some of the biomarkers involved in chronic infection include intercellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor (VEGF), endoglin, angiopoietins, and tyrosine kinase with immunoglobulin-like and EGF-like domains 1(TIE-1) [2, 13-19].

While numerous studies have been published on these markers and their correlation to HIV disease activity in adults, data on children are limited. In addition to the classical inflammatory cytokines TNF-α, IL-6, and IL-1β, other biomarkers of inflammation and those involved in endothelial activation may provide important clues about possible ongoing immune and endothelial activation despite adequate virologic control in HIV-infected infants and children.

Objective

The objective of the proposed study is to examine host proteins (“biomarkers”) that may be associated with HIV-1 disease activity among children with vertically acquired HIV-1 infection. In

particular, biomarkers of host response pathways (inflammation and endothelial activation) will be explored. The working hypothesis is that the biomarkers will provide evidence of ongoing inflammation and endothelial activation in HIV-1 infected children, despite excellent virologic control with combination antiretroviral therapy (cART).

Method

The proposed study will make use of plasma samples from HIV-infected children that have been collected as part of the CIHR-funded Early Pediatric Initiation-Canadian Child Cure Cohort Study (EPIC, principal investigator Hugo Soudeyns). To date, 150 children have been enrolled and plasma samples have been cryopreserved and are available for biomarker measurement.

Immune activation will be measured by quantifying plasma levels of sCD14, TNF, and IL-1, TNF and IL-6 will be measured in plasma by ELISA, with a detection limit of 0.1 pg/mL (R&D systems). Plasma levels of sCD14 will be quantified using human sCD14 immunoassay (R&D systems). Endothelial activation will be measured by quantifying plasma levels of ADMA, sFlt-1, IP-10, sTREM-1, Ang-1, Ang-2, soluble Tie-2, sICAM-1, soluble P-selectin, and soluble endoglin. Concentrations of these biomarkers will be measured in plasma samples using ELISA DuoSets (R&D Systems). All ELISAs will be validated prior to use, and sample dilutions will be optimized for each biomarker. ELISAs will be used according to the manufacturer's instructions. The plates will be read at 450 nm with a wavelength correction of 540 nm and concentrations interpolated using a 4-parameter logistic slope curve (GraphPad Prism v5.0). Samples from each group will be evenly distributed across ELISA plates and the researcher will be blinded to group. Statistical analysis will be done using log-transformed values of biomarker levels.

Significance

Developmental changes in immune responses with age make HIV-1 infected children an intriguing subgroup in which to explore host responses (inflammation and endothelial activation). For example, children experience a more rapid onset of HIV-1 in the absence of ART than adults [20]. Even with excellent control of viral replication with cART, a latent replication-competent HIV-1 reservoir persists, resulting in rapid virologic rebound following discontinuation of cART [21]. This latent reservoir, representing the major barrier to HIV "cure," may drive ongoing inflammation and endothelial activation, which have been shown to persist in HIV-1 infected adults [2]. By identifying biomarkers that are altered in HIV-1 infected children, we may better understand the host response to chronic HIV-1 in the pediatric population and advance the pediatric "cure" research agenda by defining correlations of HIV persistence.

References

1. Luzuriaga K. Early Combination Antiretroviral Therapy Limits HIV-1 Persistence in Children. *Annu. Rev. Med*. 2016; 67:201-13
2. Ronsholt FF, Ullum H, Katzenstein TL, Gerstoft J, Ostrowski SR. Persistent Inflammation and Endothelial Activation in HIV-1 Infected Patients after 12 Years of Antiretroviral Therapy. *PLoS One*;8:e65182.
3. Hasse B, Ledergerber B, Furrer H, Battegay M, Hirschel B et al. Morbidity and Aging in HIV-Infected Persons: The Swiss HIV Cohort Study. *Clin Infect Dis* 2011; 53: 1130–1139.

4. Fichtenbaum CJ. Inflammatory Markers Associated with Coronary Heart Disease in Persons with HIV Infection. *Curr Infect Dis Rep* 2011; 13:94-101.
5. Pentergast. CROI. 2013.
6. Cunningham-Rundles S, Kim SH, Dnistrian A, et al. Micronutrient and cytokine interaction in congenital pediatric HIV infection. *The Journal of nutrition* 1996;126:2674S-9S.
7. Johann-Liang R, O'Neill L, Cervia J, et al. Energy balance, viral burden, insulin-like growth factor-1, interleukin-6 and growth impairment in children infected with human immunodeficiency virus. *Aids* 2000;14:683-90.
8. Cervia JS, Chantry CJ, Hughes MD, et al. Associations of proinflammatory cytokine levels with lipid profiles, growth, and body composition in HIV-infected children initiating or changing antiretroviral therapy. *The Pediatric infectious disease journal* 2010;29:1118-22.
9. Jiao Y, Zhang T, Wang R, et al. Plasma IP-10 is associated with rapid disease progression in early HIV-1 infection. *Viral Immunol*;25:333-7.
10. Kutsch O, Oh J, Nath A, Benveniste EN. Induction of the chemokines interleukin-8 and IP-10 by human immunodeficiency virus type 1 tat in astrocytes. *J Virol* 2000;74:9214-21.
11. Lane BR, King SR, Bock PJ, Strieter RM, Coffey MJ, Markovitz DM. The C-X-C chemokine IP-10 stimulates HIV-1 replication. *Virology* 2003;307:122-34.
12. Denner J, Eschricht M, Lauck M, et al. Modulation of cytokine release and gene expression by the immunosuppressive domain of gp41 of HIV-1. *PLoS One*;8:e55199.
13. Ricciuto DR, dos Santos CC, Hawkes M, et al. Angiopoietin-1 and angiopoietin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. *Crit Care Med*;39:702-10.
14. Sporer B, Koedel U, Paul R, Eberle J, Arendt G, Pfister HW. Vascular endothelial growth factor (VEGF) is increased in serum, but not in cerebrospinal fluid in HIV associated CNS diseases. *Journal of neurology, neurosurgery, and psychiatry* 2004;75:298-300.
15. Shapiro NI, Yano K, Okada H, et al. A prospective, observational study of soluble FLT-1 and vascular endothelial growth factor in sepsis. *Shock* 2008;29:452-7.
16. Mitola S, Sozzani S, Luini W, et al. Tat-human immunodeficiency virus-1 induces human monocyte chemotaxis by activation of vascular endothelial growth factor receptor-1. *Blood* 1997;90:1365-72.
17. Silver KL, Conroy AL, Leke RG, et al. Circulating soluble endoglin levels in pregnant women in Cameroon and Malawi--associations with placental malaria and fetal growth restriction. *PLoS One* 2011;6:e24985.
18. Conroy AL, Lafferty EI, Lovegrove FE, et al. Whole blood angiopoietin-1 and -2 levels discriminate cerebral and severe (non-cerebral) malaria from uncomplicated malaria. *Malar J* 2009;8:295.
19. Graham SM, Rajwans N, Tapia KA, et al. A prospective study of endothelial activation biomarkers, including plasma angiopoietin-1 and angiopoietin-2, in Kenyan women initiating antiretroviral therapy. *BMC Infect Dis*;13:263.
20. Muenchhoff M, Prendergast AJ, Goulder PJR. Immunity to HIV in early life. *Front Immunol* 2014; 5; 391.
21. Martinez-Bonet M, Puertas MC, Fortuny C, et al. Establishment and Replenishment of the Viral Reservoir in Perinatally HIV-1-infected Children Initiating Very Early Antiretroviral Therapy. Clin Infect Dis 2015; doi: 10.1093/cid/civ456.