EFFECT OF HIV -1 ON CYTOTOXIC LYMPHOCYTES ISOLATED FROM A TUBERCULIN REACTIVE DONOR.

V.A. Ongaya[1, 4], M. Huante[2], M. Ferguson[3], E. Amukoye[1], J. Endsley[2]

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1. Centre for Respiratory Diseases Research, KEMRI,
2. Department of Microbiology/Immunology , University of Texas Medical Branch, Galveston, TX, USA.
3. Department of Infectious diseases University of Texas Medical Branch, Galveston, TX, USA,
4. Mount Kenya University.

Abstract

Introduction: Human immunodeficiency virus **(HIV)** infection is contributing to the re-emergence of opportunistic tuberculosis such as TB globally, and is strongly associated with the development of multi- or extensively- drug resistant tuberculosis **(MDR-, XDR-TB)**. The effects of HIV on the antibacterial activity of cytotoxic lymphocytes against mycobacteria are poorly characterized.

Objective: To determine the effect of HIV-1 on the antigen specific and innate cytotoxic/antibacterial activity of lymphocytes isolated from a tuberculin-reactive donor.

Study design: In vitro model to investigate basic concepts for how HIV predisposes people to *Mycobacterium tuberculosis* ***(M.tb).***

Setting: The work was performed at the Department of Microbiology/Immunology, University of Texas Medical Branch.

Methods: We isolated human peripheral blood mononuclear cells **(PBMC)** from a tuberculin-reactive donor and co-infected the cells *in vitro* with HIV-1 (strains Tyb and 89.6) and with the well characterized *M. bovis* Bacillus Calmette-Guérin **(BCG)**. Mock was the negative control and interleukin 15 **(IL-15)** was the positive control.

Results: Our results demonstrate that HIV-1 infection of PBMC from a healthy tuberculin-reactive donor may reduce the antimicrobial profile of cytotoxic lymphocytes activated by memory recall. The innate activation of natural killer cells was also suppressed. These effects differ with the HIV strain and the activation stimulant.

Conclusion: Understanding the effects of HIV-1 on T cell activation is essential to understanding the physiological basis for inadequate cytotoxic lymphocyte activity in HIV patients and for informed guidance of cytokine-based therapy to restore T cell function so as to boost host defense to *M.tb* or other opportunistic pathogens.

Key words: HIV, TB, cytotoxic Lymphocytes

Introduction

HIV infection is contributing to the re-emergence of opportunistic tuberculosis such as TB globally, and is strongly associated with the development of MDR- and XDR-TB. These pathogens are known to form a deadly liaison; *M.tb* infection augments HIV replication, and HIV promotes *M.tb* infection and re-activation. TB is the number 1 cause of death for people living with HIV/Aids. HIV immune compromise increases risk of infection or reactivation of TB and promotes development of drug resistant strains (1,2). TB is the largest cause of death in HIV-1 infection, having claimed an estimated one third to one half of the 30 million AIDS deaths that have occurred worldwide. Different strains of *M.tb*, the causative agent of TB, are known to modify the host immune response in a strain-specific manner (3).

Cell-mediated immunity is the major protective immune response against intracellular bacteria such as *M.tb* (4). In addition to reduction of CD4+T cell numbers, HIV alters susceptibility to pathogens via dysregulation of other cell-mediated immune mechanisms, including decreased microbicidal activity of macrophages (5). Recent studies using an *in vitro* model demonstrate that HIV infection of CD4+ T cells suppresses granulysin activation in CD8+T cells in response to IL-15 or IL-21. Granulysin is a potent antimicrobial protein contained within the granules of CTL and NK cells (6).

Methods

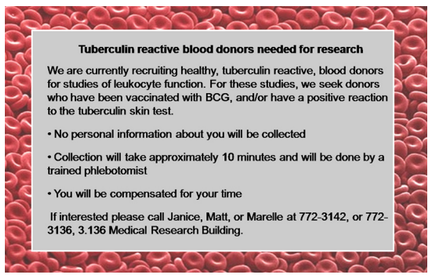

Fig 2. Recruitment of tuberculin-reactive, healthy donors (advertisement).

Methods: In these studies we used primary human peripheral blood mononuclear cells (**PBMC**) from healthy donors ages 21-49 as approved by the Institutional Review Board, University of Texas-Medical Branch. The PBMC were used to determine the *in vitro* effects of HIV-1 (strains Tyb and 89.6) on antigen-specific (memory) recall to PPD (purified protein derivative) or BCG by peripheral blood T cells of healthy tuberculin skin test positive individuals. The innate response of natural killer (**NK**) cells was also determined. Antigen-specific T cells activation was assessed by using multivariate flow cytometry.

Staining panel included:

CD4+ -Pacific blue	Granulysin-Fitc/488
CD8+ - Percpcy5.5	CD107a- PE-Cy5

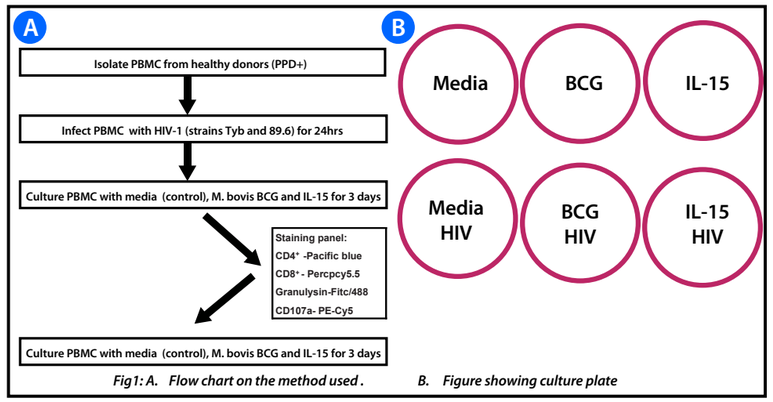

Fig1: A. Flow chart on the method used . B. Figure showing culture plate

Results

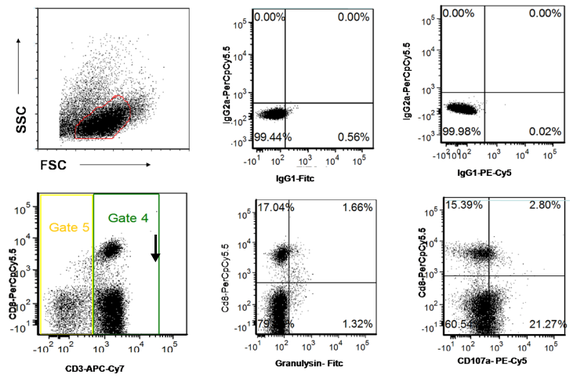

Fig 3: Analysis template for evaluating activation of effector function in subsets of cytotoxic lymphocytes. PBMC were activated with control (BSA) or IL-15 (15 ng/ml) for 3 days and surface expression of CD3, CD4, CD8, CD56, CD107a, and intracellular granulysin was detected by flow cytometry. Shown is flow cytometric analysis of PMBCs, side scatter and forward scatter characteristics of isolated cells, gating strategy for analysis of CD3-CD56+ cells, and quadrant settings to account for background fluorescence using isotyped-matched non-specific monoclonal antibodies and representative plots of expression of granulysin and CD107a by CD3+CD8+ T cells.

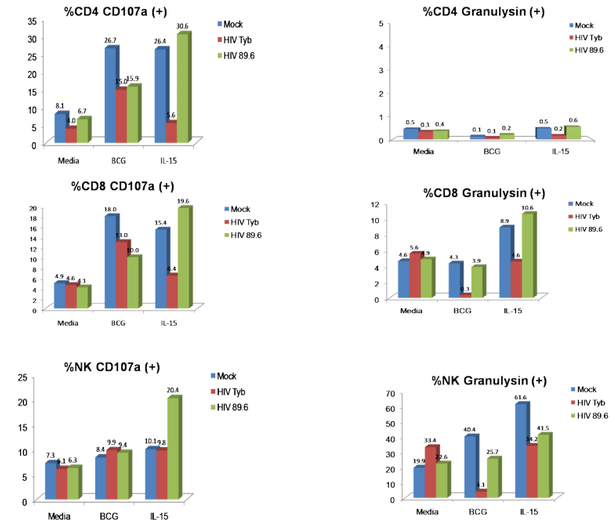

Fig 4: HIV-1 suppresses expression of molecules important for T cell and NK cell antibacterial function. Flow cytometry was used to analyze intracellular expression of granulysin within CD3+CD4+ or CD3+CD8+ T cells, or CD3-CD56+ NK cells. PBMC from healthy donors were infected with HIV-1 Tyb (X4) or 89.6 (dual tropic) for 24 hrs before addition of BCG (1 MOI) or rIL-15 (15 ng/ml) for 3 days. Samples were collected on a BD FACS Fortessa using FACSDIVA software and analyzed using FCS Express version 3.

Discussion

- ★ CD4+ and CD8 + T cells increased expression of CD107 after exposure to antigen (BCG) or IL-15.
- ★ HIV-1 isolates Tyb and 89.6 suppressed CD4+ and CD8+ T cell activation of CD107a by antigen (BCG), while only Tyb affected the IL-15 response.
- ★ NK cells showed the highest expression of granulysin, with innate activation by BCG as well as cytokine activation by IL-15 observed.
- ★ Activation of granulysin expression by CD8+ T cells and NK cells was suppressed by HIV-1, with differences in effects observed among the two HIV isolates.
- ★ These results suggest that HIV-1 infection of PBMC may reduce the antimicrobial profile and cytotoxicity of activated cytotoxic lymphocytes, and these effects may differ with the HIV strain and the activation stimulant. Further studies are needed to determine the importance of these effects in HIV/*M.tb* co-infection.

Conclusion

- ★ Understanding the effects of HIV-1 on T cell activation is essential to understanding the physiological basis for inadequate cytotoxic lymphocyte activity in HIV patients and for informed guidance of cytokine-based therapy to restore T cell function so as to boost host defense to *M.tb*.

Future Research Direction/Recommendation

- ★ Characterize cytotoxic lymphocyte (T cell and NK cell) defects in HIV and HIV/*M.tb* co-infected patients.
- ★ Identify molecular mechanisms whereby HIV or HIV proteins may suppress T cell activation.
- ★ Investigate ways to restore cytotoxic/antibacterial lymphocyte function through immune modulation.

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