In HIV Infected Patients Killing of Latently HIV-Infected CD4 T Cells by Autologous CD8 T Cells is Modulated by Nef.

Ziv Sevilya1,2, Ehud Chorin2, Orit Gal-Garber1, Einaït Zelinger4, Dan Turner2,3, Boaz Avidor1, Gideon Berke3 and David Hassin1,2,3,6

1Internal medicine department A, Assuta Ashdod Medical Center 2Crusaid Kohler AIDS center, Tel-Aviv Sourasky Medical Center; 3Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv; 4Interdepartmental Equipment Facility, Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University, Rehovot; 5Department of Immunology, Weizmann Institute of Science, Rehovot; 6Faculty of Health Sciences, Ben Gurion University of the Negev; Israel

Abstract
Background: The PBMC of HIV-infected patients contain HIV-specific CD8 T cells and their potential targets, CD4 T cells latently infected by HIV. The role of HIV-specific CD8 T cells in the course of HIV infection and the way they affect the virus that resides in the latent reservoir, the resting memory CD4 T cells, is unknown. The association between HIV Nef protein and the cellular ASK1 kinase protects the HIV-infected CD4 T cells from killing by CD8 T cells.

Methods: CD8 and autologous CD4 T cells procured from PBMC of acute, chronic uninfected, treated and AIDS patients were isolated by magnetic beads and co-incubated. Resting memory CD4 T cells (CD25+, CD69+ and HLA-DR−) were isolated from activated CD4 T cells using a two-step bead depleting purification procedure. Formation of CD8-CD4 T-cell conjugates was observed by fluorescence microscopy and in situ PCR of HIV LTR DNA. Both conjugation and apoptosis were observed and quantified by imaging flow cytometry (ImageStream) using anti-human activated caspase 3 antibody and TUNEL assay. Formation of immunological synapse was observed by using anti-Perforin, anti- γ-tubulin, and anti-LCK antibodies.

Results: Following co-incubation we observed that CD8 T cells conjugate with and induce apoptosis of autologous CD4 T cells. In patients with acute infection or AIDS the conjugation activity and apoptosis were much higher compared to chronic HIV-infected patients. In patients on Anti-Retroviral Therapy (ART) low grade conjugation of CD4 T cells was observed by fluorescence microscopy (2.3±0.3 %), by in situ PCR of HIV DNA (3±0.6 %) and by ImageStream analysis (2.5±0.5 %). After co-incubation with autologous CD8 T cells 2.1±0.4 % of the CD4 T cells procured from patients on ART were undergoing apoptosis. Resting memory CD4 T cells were conjugated (1.9±0.3 %) and killed (2.2±0.3 %) by autologous CD8 T cells. Delivering a peptide that interferes with the Nef-ASK1 interaction, into the CD4 T cells, resulted in two-fold enhancement of their apoptosis by the autologous CD8 T cells (from 2.1±0.5 % to 4.0±0.4 %), with no effect on conjugation.

Conclusions: CD8 T cells conjugate with and kill HIV-infected CD4 T cells throughout the course of HIV infection. We propose that Nef inhibition may result in the elimination of the latent reservoir CD4 T cells by CD8 T cells.

Conjugation and immunological synapse formation between CD8 and CD4 T cells procured from patients on ART

Anti-CD8 T cells (APC red), Anti CD4 T cells (PE Cy7 green), ImageStream

Conjugation and apoptosis of CD4 T cells by autologous CD8 T cells procured from patients on ART

ImageStream images of TUNEL and activated caspase-3 positive CD4 T cells treated with ASK1 peptide conjugated with Nef.

The resting memory CD4 T cells can be recognized and undergo apoptosis by autologous CD8 T cells in patients on ART

Resting memory CD4 T cells (CD25+, CD69+ and HLA-DR−) isolated from activated CD4 T cells using a two-step bead depletion purification procedure.

Inhibition of HIV Nef interaction using ASK1 aminopeptide sequence 152-DEVGEANNS (Kumar et al. 2019)

CD4 and CD8 T cells were procured from PBMC of five HIV-1 infected patients on ART and mixed and incubated. Fixed cells were labelled as indicated and analyzed by ImageStream.

Conclusions:
1. CD8 T cells conjugate with and kill HIV infected CD4 T cells, including resting memory CD4 T cells, throughout the course of HIV infection.
2. HIV Nef protein interacts with ASK1, thus protecting HIV-infected cells from CD8 T cells killing.
3. We propose that Nef inhibition may result in the elimination of the latent reservoir CD4 T cells by CD8 T cells.