

ABSTRACT FORM
Annual IDM Research Day
September 17, 2008

Abstract Title:

Abstract Title: MECHANISTIC STUDIES ON A CD8 SUPPRESSIVE FACTOR THAT INHIBITS HIV-1 REPLICATION

Authors and Affiliation:

Varsha Shridhar, B.Tech*, Ashwin Tumne, PhD[#], Yue Chen, PhD[#] and Phalguni Gupta, PhD[#]

Text:

*: Pittsburgh Retrovirology Laboratory, Molecular Virology and Microbiology Program, University of Pittsburgh School of Medicine, Infectious Diseases and Microbiology, #:Pittsburgh Retrovirology Laboratory, University of Pittsburgh Graduate School of Public Health

CATEGORY OF EMPHASIS: Bioscience

Abstract

Introduction: CD8+ cells from HIV infected individuals can inhibit viral replication in acutely infected CD4+ cells in a non-cytolytic manner by suppressing transcription from the HIV promoter, the long terminal repeat (LTR). The mechanism of non-cytolytic anti-viral action is strongest when there is cell to cell contact between the CD8+ and the CD4+ cells, though CD8 culture supernatant has also been found to significantly (>80%) suppress HIV transcription.

In our efforts to study the mechanism of suppression and identify the factor responsible, we recently detected a membrane-bound HIV-1 suppressing activity secreted as 30-100 nm sized exosomes from transformed CD8+ T cells. The exosomes were found to be a good model for these studies.

Objective: To elucidate the process by which the suppressive factor produced by CD8 T cells, and present on CD8 exosomes, mediates its effects on HIV LTR to inhibit transcription.

Methods: The interaction between the exosomes and the target cells was studied by confocal microscopy, using Cy5 (red) labeled exosomes and PKH67 (green) labeled target cells. Cell lines knocked out for individual signaling molecules were used to identify intracellular signaling molecules. To localize the region on the LTR crucial for suppression of transcription, deletion mutant analyses of the LTR were performed.

Results and Discussion: Confocal microscopy shows that the interaction of exosomes with the cells is restricted to the surface and occurs rapidly (within 10 minutes of addition). However, significant suppression of LTR driven transcription is observed only after 12-16 hours. This suggests the possible production of an intermediate protein. Studies using the knock-out cell lines suggest the involvement of STAT1 in this process. Experiments using deletion mutants of the HIV LTR suggest that the region necessary for suppression is the TAR (trans-activation responsive) region. This is very intriguing because the TAR region is not only crucial for transcription of the HIV genes, but is also one of the conserved regions in the HIV genome. This could explain why the suppressive factor is effective regardless of the subtype or the strain of the virus. Thus, elucidation of the molecular mechanisms of suppressive factor action has great significance both in the fields of viral immunology as well as therapy.

CATEGORY OF EMPHASIS

(check all that apply)

Bioscience

Education/Prevention