Innate mucosal Serpin B1 inhibits lates stages of HIV life cycle and reduces cellular proliferation

Aboud L.[1,2], Jayappa, KD.[2], Kimani J.[5], Yao X.[2], Plummer FA[2,4], Burgener A[1,2,4], Ball TB[1,2,3,4]

[1]J.C Wilt Infectious Disease Research Centre, Winnipeg, Manitoba [2]Department of Medical Microbiology and [3]Immunology, University of Manitoba, [4]Winnipeg, Canada. National HIV and Retrovirology Laboratory, Public Health Agency of Canada, Canada. [5]Department of Medical Microbiology, University of Nairobi, Kenya.

Introduction

Females, acquiring HIV through heterosexual transmission, bear an increasingly heavy burden in the number of annual HIV infections (~60%).

An individual's risk of becoming infected with HIV is dependent on numerous factors, including the biological fluids produced by the genital mucosa, which contain a plethora of both anti-viral and pro-viral factors.

Various antiproteases, including specific members of the serpin family, have been identified as up-regulated in the cervico-vaginal lavage (CVL) of HIV-Exposed Sero-Negative (HESN) women in Nairobi, Kenya.

Serine Proteases are secreted by immune cells including cytotoxic T cells and neutrophils and function to kill invading pathogens through triggering of the complement system and induction of inflammatory mediator responses.

Serpins (Serine Protease Inhibitors) are found in epithelial cells as well as many immune cells and function to regulate inflammation and tissue development as well as defense against invading pathogens, through regulation of serine, and specific cysteine, proteases.

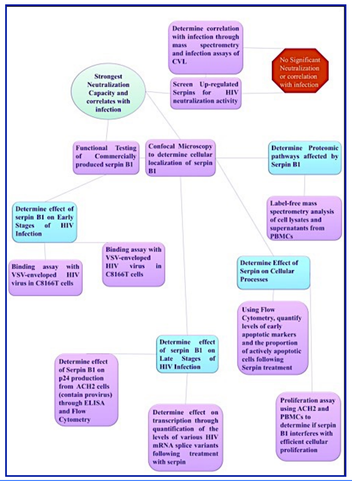
The observed up-regulation of numerous serpins within the CVL of HESN women may contribute to their protective phenotype through control of immune response and hence the degree of inflammation within the female genital tract, resulting in a reduced immune activation state within potential HIV target cells.

Specific serpins have also demonstrated direct HIV inhibitory activity (Serpin A1, Serpin C1). It is thus, reasonable to hypothesize that other serpins exhibit similar effects and may prove to be novel candidates for future HIV-1 microbicides.

Hypothesis

Specific serpins identified as up-regulated within the CVL of HESN women, will exhibit HIV-1 neutralization activity through mechanisms that are both directly targeted against HIV-1 as well through indirect cellular mechanisms, including regulation of local inflammation and cellular activation

Methodology

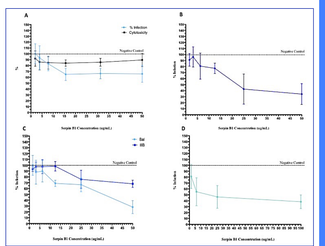


Results

Serpin B1 treated cells results in decreased levels of HIV infection

Figure 1: Serpin B1 Inhibits HIV in multiple cell lines and a tissue explant model

Serpin B1 inhibited HIV infection within TZM-bl and C8166T cell lines and within PBMCs infected with both an R-tropic and X-tropic HIV virus as well as in a cervical-explant tissue model.



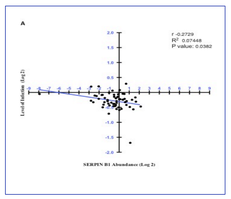
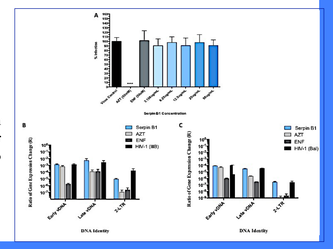


Figure 2: Serpin B1 is correlated with the degree of HIV inhibition exhibited by CVL of Nairobi commercial sex workers

Serpin B1 does not interfere with early stages of the HIV life cycle

Figure 3: Serpin B1 does not alter the level of HIV DNA within infected cells

Serpin B1 does not interfere with proper reverse transcription, nuclear import or integration of HIV DNA into host DNA.



Serpin B1 interferes with post-transcriptional stages of HIV life cycle

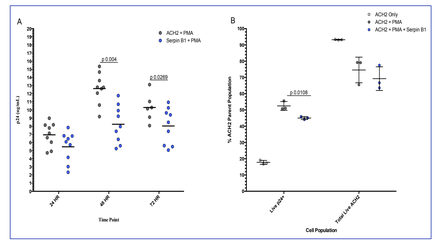


Figure 4: SerpinB1 reduces extracellular and intracellular levels of p24 production in ACH2 cell line

Serpin B1 reduced the amount of virus produced by ACH2 cells, which have a provirus integrated into their genome. Both extracellular levels and intracellular levels of p24, were reduced following treated with serpin.

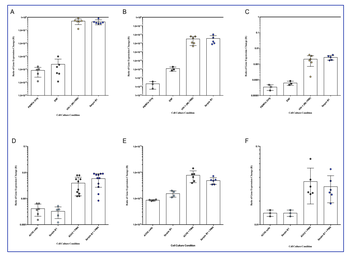


Figure 5: Serpin B1 does not alter levels of HIV mRNA splice variants in PBMCs or ACH2 cells

ACH2 cells and PBMCs treated with serpin B1 did not exhibit altered levels of unspliced, single-spliced or multi-spliced mRNA splice variants compared to negative controls. This suggests that proper transcription is taking place.

Cellular effects may be causing observed reduction in HIV infection

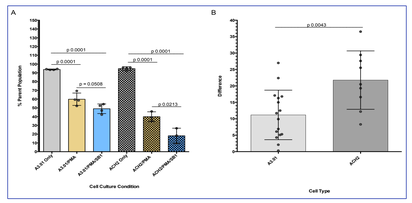


Figure 6: Serpin B1 reduces cellular proliferation significantly in ACH2 HIV infected cells

Following flow cytometry proliferation analysis of ACH2 cells compared to their parent, uninfected, A3.01 cell line, it was clear that while serpin reduces the level of cellular proliferation in both cell lines, it did so to a much more significant level within the HIV infected ACH2 cell line.

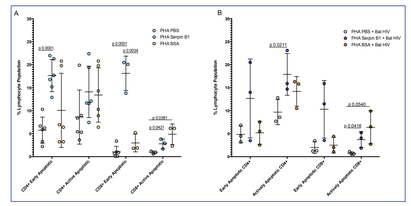


Figure 7: Serpin B1 reduces the number of actively apoptotic ACH2 and A3.01 cells and increases the number of early apoptotic cells

When PBMCs are treated with Serpin B1 there is an increase in the number of early apoptotic cells in CD4 and CD8+ T cells.

Proteomic profile of PBMC cell lysates treated with Serpin B1

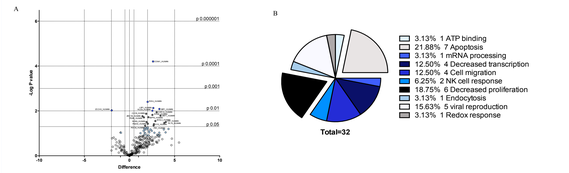


Figure 8: Biological functions associated with Serpin B1 treated, HIV-infected PBMC lysates

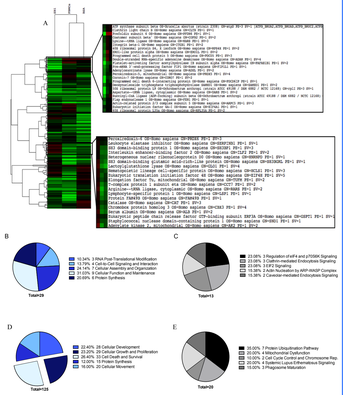


Figure 9: Cluster analysis of differentially expressed proteins in cell lysates treated with Serpin B1

A: dendogram, B: Cellular and molecular functions for under-expressed proteins in cell lysates of Serpin B1 treated PBMCs, C: canonical pathways associated with under-expressed proteins, D: Cellular and molecular functions associated with over-expressed proteins in the cell lysates of Serpin B1 treated PBMCs, E: canonical pathways of over-expressed proteins.

Conclusions

Naturally occurring over-abundant serpins within the FGT of HESN women are capable of inhibiting efficient HIV infection in numerous cell lines and in a tissue explant model.

Serpin B1 does not have a direct effect in early stages of the HIV life cycle but rather in steps post-transcriptionally.

Serpin B1 interferes with efficient cellular proliferation possibly through induction of a "quieting" of the cells by inducing early apoptotic pathways, or through reduction in protein translation/increased targeting of proteins for ubiquitination.

This serpin may be useful in conjunction with other agents in a novel microbicide.

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