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

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**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 cervicovaginal lavage (CVL) of HIV-Exposed Sero-Negative (HESN) women in Nairobi, Kenya.

Serine Proteases are secreted by immune cells including cytototoxic 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**

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

Serpin B1 reduces the amount of viral DNA produced by ACH2 cell line

Serpin B1 treated HIV-infected PBMC lysates

Figure 2: Serpin B1 reduces cellular proliferation significantly in ACH2 HIV infected cells

Serpin B1 reduces the number of actively apoptotic ACH2 and A549 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

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 “quitting” 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.

**Acknowledgements**

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**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-Bi and C8166 cell lines and within PBMCs infected with both an R5ropic and X tropic HIV virus as well as in a cervical explant tissue model.

Figure 3: Serpin B1 does not interfere with early stages of the HIV life cycle

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Figure 4: Serpin B1 does not alter the level of HIV DNA within infected cells

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

**Figure 5:** Serpin B1 does not alter the level of HIV DNA within infected cells

Following flow cytometry proliferation analysis of ACH2 cells compared to their parent, uninfected, A549 cell line, it was clear that while serpins reduce 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.

Serpin B1 reduces cellular proliferation significantly in ACH2 HIV infected cells

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

PBMCs Only

Serpin B1 does not exhibit induction of early apoptosis

Serpin B1 treated, HIV-infected PBMC lysates

**Figure 7:** Serpin B1 reduces the number of actively apoptotic ACH2 and A549 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

**Figure 8:** Biological factors associated with Serpin B1 treated HIV-infected PBMC lysates

PBMCs Only

Serpin B1 does not exhibit induction of early apoptosis

Serpin B1 treated, HIV-infected PBMC lysates

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