Cell-Associated HIV Transmission: Does Seminal Plasma Affect the Viability and Migration of Macrophages?

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Abstract

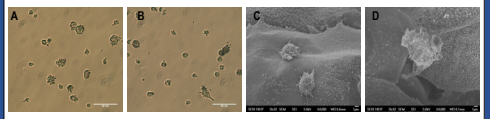
PROBLEM: The role of seminal plasma (SP) in the transmission of HIV-1 remains controversial: both facilitatory and inhibitory effects have been described. Our laboratory has championed the concept that HIV-infected WBCs in semen are "Trojan Horse" vectors of HIV transmission. In this model, intracellular virus is protected from inactivating factors in the genital environment, and cell-cell transmission of HIV via intercellular synapses is highly efficient. Because macrophages are the most abundant classical HIV host cell in semen, and HIV-infected macrophages have been isolated from semen of HIV-infected men, they are likely principal mediators of cell-associated HIV transmission. However, little is known about the effect of seminal plasma on the viability and migratory potential of macrophages.

MATERIALS AND METHODS: Macrophages used in the study were: 1) seminal macrophages from semen of normal donors, 2) PMA-activated U937 cells and, 3) monocyte-derived macrophages (MDM) obtained from normal human donor blood by differentiation of monocytes in medium containing 10% human serum for 10 days. U937 cells or MDMs were treated with seminal plasma either continuously for up to 24 hours or pulsed with seminal plasma for 2 hours after which the seminal plasma was replaced with tissue culture medium. Cell viability was assessed by trypan blue exclusion, MTT assay and by flow-cytometry using live-dead dual staining (Live stain: CMFDA or Calcein-AM and dead stain: ethidium homodimer). For cell migration studies, CMFDA-stained macrophages were suspended in seminal plasma or culture medium and applied to the apical surface of EpiVaginal TM tissue (MatTek Corp); penetration into tissue was monitored by confocal microscopy. For some experiments, MDMs were infected with an R5-tropic GFP-expressing HIV construct 3 days prior to tissue infiltration. Analysis: Data were analyzed using ANOVA with Fisher's PLSD post hoc comparisons. Infiltration data was analyzed using ImageJ software.

RESULTS: Seminal macrophages adhered to glass slides and remained viable (excluded trypan blue) for >24 hours. U937 cells and MDMs remained >50% viable for at least 8 hours after short-term or continuous exposure to seminal plasma. Seminal macrophages and seminal plasma-exposed MDMs were able to adhere to and infiltrate vaginal epithelial tissue. Further, GFP+ HIV-infected leukocytes were similarly found at depths >150um below the epithelial surface.

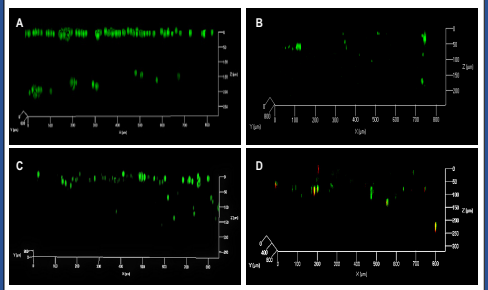
CONCLUSIONS: Macrophages remain viable and maintain adherence and migratory function after short-term exposure to seminal plasma and infection with HIV. These data support a role for HIV-infected macrophages in the sexual transmission of HIV.

Figure 1: Adherence and infiltration of macrophages



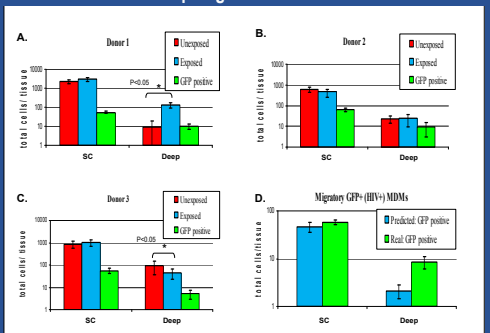
Seminal macrophages adhere to glass slide and exclude trypan blue at (A) 3 hours and (B) 24 hours. Scanning electron microscopy images of macrophages (C) adhering to the surface, and (D) infiltrating the vaginal epithelium (VEC model).

Figure 2: Uninfected and HIV-infected leukocytes migrate into TNF-α treated EpiVaginal™ Tissue



EpiVaginal™ tissue was pretreated for 24 hrs with TNF-α. Leukocytes were labeled with CellTracker green CMFDA and were applied to the luminal surface of EpiVaginal tissue. After 2 hours of infiltration, a Z-stack was taken at 5-mm intervals through the tissue obtained using deconvolution microscopy. Reconstructions shown for: (A) PMA activated U937 cells, (B) seminal mononuclear leukocytes, (C) monocyte derived macrophages, and (D) monocyte derived macrophages infected with m-Cherry-expressing HIV construct

Figure 3: HIV-infected leukocytes migrate into TNF-α-treated EpiVaginal™ Tissue



(A-C). Infiltration of HIV uninfected and infected MDMs from 3 individual donors into the stratum corneum (SC) or deep epithelial layers (>90um). Unexposed = not exposed to HIV (Control), Exposed = MDMs exposed to HIV, and GFP positive = the subset of HIV-exposed MDMs productively infected with HIV (GFP positive). (D) Composite data from the 3 donors showing predicted GFP$^+$ versus real GFP$^+$ cell counts.

Figure 4-I: PMA-activated U937 cells withstand short-term exposure to seminal plasma, but are killed with continuous exposure to seminal plasma (≥ 8hrs)

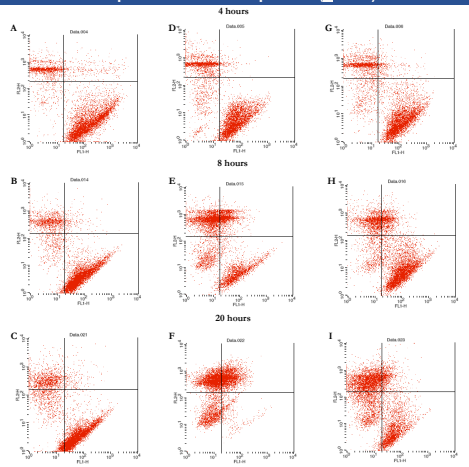
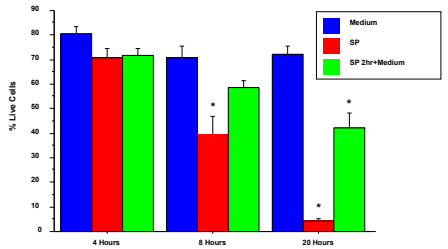


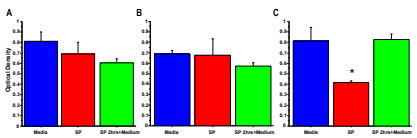
Fig 4-I: U937 monocytes underwent a 36-hour activation to stimulate differentiation into macrophages. Cells were then treated with A, B, C= medium, D, E, F= seminal plasma and G, H, I = 2-hour pulse with seminal plasma and then medium. Flow cytometry was then performed after labeling the cells with Calcein AM or ethidium homodimer. Y-axis represents dead cells labeled with ethidium. The X-axis shows Calcein labeled live cells.

Figure 4-II.



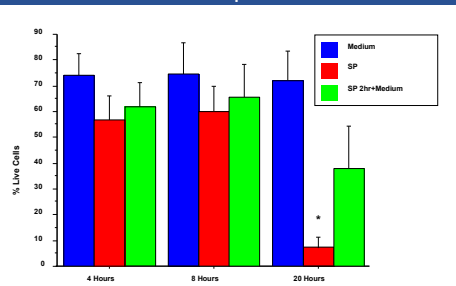
Data from Figure 4 graphed as % live cells after treatment with medium, seminal plasma or 2-hour pulse with seminal plasma followed by medium over a time course of 4, 8 and 24 hours. * p<0.01

Figure 5: Short exposure to seminal plasma does not affect metabolic activity of activated U937 cells in the MTT assay



PMA-activated U937 cells were treated with medium, continuous seminal plasma or 2-hour pulse with seminal plasma and then medium. MTT assay was then performed

Figure 6: MDMs withstand short-term exposure to seminal plasma, but are killed with prolonged continuous exposure to seminal plasma



Percentage of live cells after treatment with medium, seminal plasma or 2-hour pulse with seminal plasma followed by medium over a time course of 4, 8 and 24 hours. * p<0.01

Conclusions

1. Macrophages (activated U937 cells and MDMs) maintain adherence, viability and migratory function after short-term/ physiological exposure to seminal plasma.
2. Mononuclear leukocytes isolated from fresh semen remain viable for >24 hours, and can adhere to and infiltrate EpiVaginal tissue.
3. Preliminary data indicate that HIV-infected MDMs infiltrate deeper into vaginal epithelial tissue than uninfected MDMs.
4. These data support a role for HIV-infected macrophages in the sexual transmission of HIV.

References

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