Tunneling Nanotubes: A Novel Target to Prevent HIV Reactivation

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BACKGROUND

HIV is well-known to be transmitted through cell-free viral particles, largely due to its requirement for CD4 and CCR5/CXCR4 receptors for cell entry. However, the mechanisms underlying systemic HIV spread following receptor-mediated entry or latency reactivation, particularly when the levels of cell-free virus are low to undetectable, remain unclear. Here, we demonstrate that tunneling nanotubes (TNTs), are induced during early stages of infection or reactivation to facilitate viral spread through an intercellular pathway independent of CD4 and CCR5/CXCR4 receptors, until soluble virus becomes predominant. TNTs are long processes that allow communication between the cytoplasm of two or more connected cells. TNTs differ from filopodium by length, cellular origin, and induction mechanisms. TNT's major component is F-actin, and in HIV, TNTs can reach over 500-µm *in vivo* and *in vitro*, but their cargo and function have recently been identified and explored. TNTs are only expressed in disease conditions, such as cancer, HIV infection, and reactivation. Furthermore, TNT formation triggered by HIV infection or latency reactivation prevents immune recognition, gp120 antibody neutralization, and virus-associated cytotoxicity. Our data indicate that TNTs represent a mechanism of targeted infection and viral amplification, providing a promising therapeutic target to complement HIV cure strategies.

METHODS

1) To examine the time course of TNT formation in response to HIV infection (HIV_{ADA}, $HIV_{JR\text{-}CSF}$, or HIV_{BaL}, MOI, 0.01), primary human $CD4^+$ T lymphocytes and macrophages as well as PBMCs from people living with HIV (PLWH) under a successful ART regimen were cultured, and TNT formation was evaluated by live-cell imaging, with image acquisition every 30 sec or 1 min up to 28 days post-infection, in the presence and absence of ART treatment (100 nM efavirenz, 180 nM zidovudine, and 200 nM raltegravir). In parallel, cell cultures were fixed and stained for nuclei, HIV-p24, F-actin, and the TNT marker TTHY1 to quantify TNT formation and viral protein expression and distribution after 3, 6, 9, 12-, 14-, 21-, and 28-days post-infection.
2) To quantify TNT-mediated transfer of viral proteins and RNA we use a co-culture model between U87 cells transfected with CD4 and CCR5 (U87CD4CCR5), which are susceptible to extracellular HIV infection, and U87MG cells which cannot be infected by extracellular HIV because they lack expression of CD4 and CCR5 receptors (U87MG), but they are competent … replicate the virus when are transfected and can form TNTs with U87CD4CCR5 cells. Using this model, we did laser capture microdissection, and subsequent RNAseq and proteomics, as well as immunofluorescence staining.

Tunneling Nanotubes (TNTs) structures are induced during initial HIV infection and after latency reactivation. TNT communication is essential to facilitate HIV spread when the cell-free virus is not abundant. TNT formation helps to protect the infected cells from immune recognition and apoptosis.

RESULTS

- TNTs formation is induced after CD4 and CCR5/CXCR4-mediated entry and infection, as well as after ART interruption and reactivation.
- TNTs structures transport infectious cargo between HIV infected and uninfected cells.
- TNT formation prevents HIV toxicity, immune recognition, and the efficacy of neutralizing antibodies.
- Blocking TNTs prevents viral spread/replication and enhances viral/immune-mediated cytotoxicity.

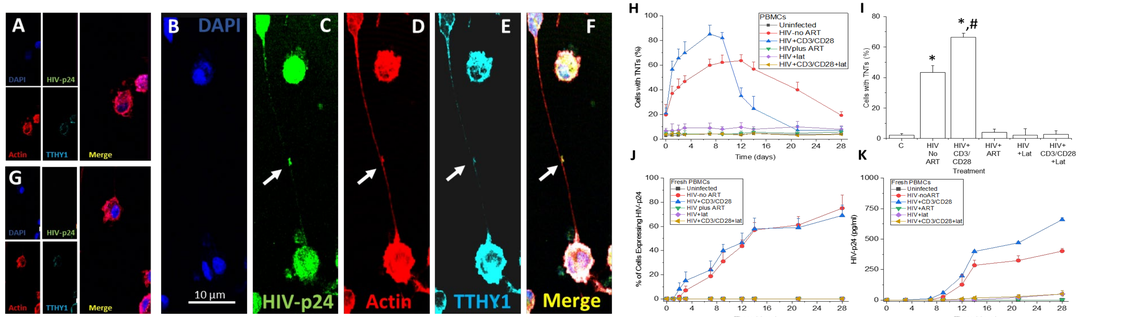


Fig. 1. PBMCs from PLWH under active ART and effective viral suppression were examined for TNT formation and spread of infection.

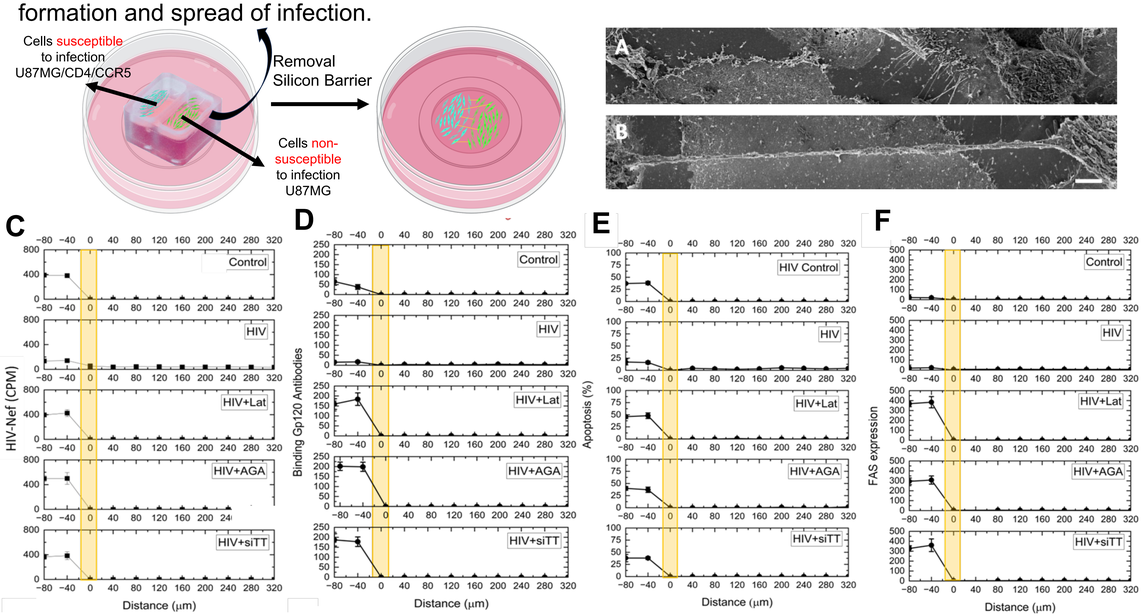


Fig. 2. Blocking TNT formation results in intracellular viral protein accumulation, enhanced neutralizing viral recognition, and immune activation, as well as apoptosis.

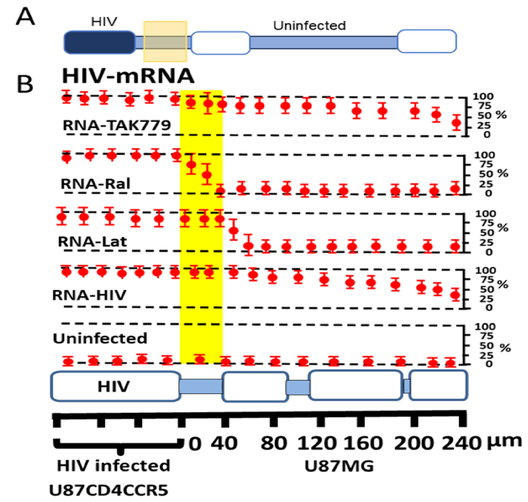


Fig. 3. TNTs are essential to amplify the infection by the transfer of viral RNA.

CONCLUSIONS

In conclusion, TNTs are induced by HIV infection and reactivation to enhance viral spread while evading immune detection, apoptosis, and neutralizing antibodies critical for establishing and maintaining infection. TNTs deliver infectious cargo, not whole virions, enabling viral RNA to integrate into targeted cells. The exquisite selectivity of TNT formation and associated transport enhances viral spread 100-1000 times faster than soluble virus alone. Targeting TNTs offers a promising therapeutic strategy to curb viral amplification and spread, advancing efforts toward an HIV cure.

ADDITIONAL KEY INFORMATION

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PLAIN LANGUAGE SUMMARY

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