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Article Addendum

Tunneling nanotubes (TNT)

A potential mechanism for intercellular HIV trafficking

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Cell-to-cell communication coordinates the development of multicellular systems, and is mediated by soluble factors, gap junctions and the recently described tunneling nanotubes (TNT). Both TNT and gap junctions facilitate the transfer of intracellular mediators between the cytoplasm of connected cells. We recently described that HIV induced the formation of TNT in human primary macrophages in correlation with viral replication. Based on these results we hypothesized that during HIV infection, TNTs are hijacked by HIV to spread infection. TNT like structures may be a novel mechanism of amplification of HIV infection. Our findings and those of others require further investigation to identify the specific mechanisms by which pathogens use TNT.

Tunneling nanotubes (TNT) and gap junctions are the only two described communication systems that allow exchange of cytoplasmatic factors through direct contact between the cytoplasm of connected cells. These communication systems coordinate biological processes, including development, metabolism, homeostasis and the immune response.[1-6] The major differences between TNT and gap junctions are the distances reached and the sizes of the molecules transferred. TNT mediate long-range communication through extended processes, while gap junctions facilitate close cell-to-cell communication. Gap junctions allow the trafficking of small molecules, up to 1.2 kDa,[6] while TNT allow the exchange of small molecules, organelles and vesicles.[2]

Our recent report, using primary human macrophages and HIV, suggested that TNT could be "hijacked" for the virus to spread between connected cells during the periods of high viral replication. HIV infection of macrophages enhanced the numbers of TNT and more infected cells expressed TNT, suggesting that HIV induced the expression or stability of these processes to allow viral spread through this mechanism.[1] In the past year it has been shown that the virus utilized TNT-like structures to spread infection between connected T cells[7] and we demonstrated HIV-p24 in TNT of HIV infected macrophages.[1] We and others proposed that HIV, by using this pathway of spread, will infect cells more efficiently without entering the extracellular compartment, reducing viral exposure to natural anti-viral activities as well as to potential antiviral drugs. In agreement with this, it has been demonstrated that viral infections, including HIV, are increased by several orders of magnitude when cell to cell contact is involved, suggesting that direct actin cytoskeleton interactions between connected cells allow efficient viral spread.[8,9]

Although there is a basal level of TNT expression by cells under normal tissue culture conditions, the signals that guide the formation of TNT are unknown. However, re-examination of reports in the existing literature show that there are published descriptions of increased formation of TNT like structures in inflammatory conditions. In in vitro pathological conditions the formation of TNT-like structures has been observed after infection with *Listeria monocytogenes* and *mycobacterium bovis*,[10-12] in astrocytes treated with H_2O_2,[13] microglia activated with PMA and calcium ionophore,[14] monocyte/macrophages treated with LPS plus IFNγ,[15] mouse neuronal cells infected with exogenous prion protein (PrP)[16] and more recently in lymphocytes infected with HIV[7] and in human macrophages infected with HIV.[1] In vivo, TNT like structures have been observed in Drosophila,[17,18] between immune cells in lymph nodes (reviewed in refs. 2 and 3), in the dendritic cells (DC) of the gut[19,20] and in the MHC class II$^+$ cells in the mouse cornea.[21] Interestingly, viruses, such as African swine fever, Ebola, Herpes Simplex, Marburg filoviruses and Poxvirus Vaccinia encode viral factors or alter cell activation to induce formation of filopodia structures to allow viral trafficking between the extracellular matrix or environment into cells,[22-28] suggesting that viruses are able to use filopodia and TNT like structures to improve viral spread.

We still have a limited understanding of TNT function and turnover. It is unclear whether all TNTs are similar in length, internal size, permeability, transport capability (internal and external transport), and signaling properties. Our studies in human macrophages identified two distinct TNT morphologies that are altered by HIV infection, referred to as short- and long-range TNT. Both types of TNT, as well as filopodia, can coexist independently in different regions of the same cell, suggesting a cellular compartmentalization for the formation and transport through these processes in each cell.[1]

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However, the specific function of each type of process, and whether these processes have differential permeability or transport properties remains unclear. In our system, filopodia did not connect cells. A recent report supports the idea of different types of TNT on different cells, showing that TNT in lymphocytes are almost impermeable to calcium,[7] in contrast to the high permeability to calcium of TNT observed in dendritic and THP-1 cells.[29] TNT in other systems allows transport of mitochondria and vesicles, suggesting that the internal pore size is large enough for the trafficking of these organelles (reviewed in refs. 2 and 30). The point that mitochondria can be exchanged between TNT connected cells is extremely important because this could be one of the first demonstrations of transfer of genetic material between non-dividing cells, suggesting that at least mitochondrial DNA is not cell type exclusive and can be shared between several cell types connected by TNT. Although it is still unclear whether multiple types of TNT exist or represent different maturation stages of the same processes, the potential for the transfer of organelles and changes in signaling opens a new era in understanding the cell as a unique entity.

Several groups have proposed at least two models to explain the varied types of TNT, based on cell type, permeability, signaling capabilities, length and function. The first model proposes a tube generated from one or both cells involved, resulting in fusion of membranes, leaving a continuous tube between the connected cells, allowing the transfer of molecules between the connected cells. The second model proposes that the processes do not form a tube, but rather are composed of adhesion molecules and other molecules involved in signaling that aggregate in the tip of the TNT, like a synapse, to coordinate intercellular communication. Based on the multiple lengths, cell types and potential function of these TNT in normal and in pathologic conditions, we believe that both proposed TNT systems may exist and require extensive investigation to determine the function of these TNT structures.

In conclusion, we propose that TNT processes may help HIV infection and other pathogens to spread more efficiently while avoiding extracellular anti-viral responses, increasing the chance that small populations of infected macrophages or T-cells will spread infection to a large number of other cells. An understanding of the role of this new communication system in normal and pathological conditions may open new potential therapeutic opportunities to target HIV infection and replication.

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