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Large Syncytia in Lymph Nodes Induced by CCR5-Tropic HIV-1

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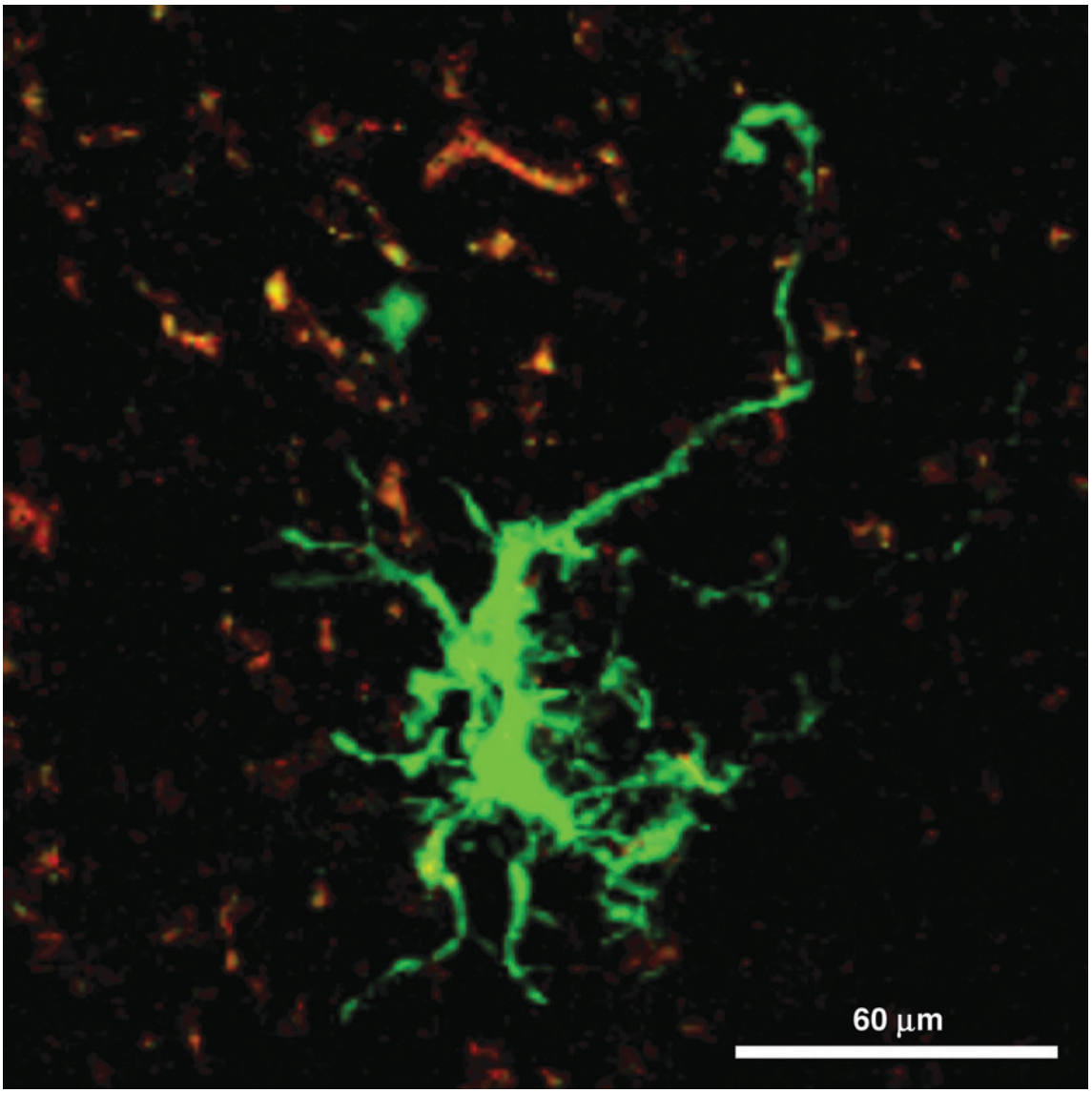

FIG. 1. Large syncytium with multiple dendritic extensions in the lymph node of a BLT humanized NOD-SCID mice. The footpad was injected with 1×10^5 IU of HIV-GFP and the draining popliteal lymph node was prepared for intravital microscopy at day 6 postinfection. The micrograph of HIV-infected cells ($EGFP^+$; *green*) is a maximum intensity projection of 11 z-stacks, spaced 4 μm apart (total thickness of 40 μm). *Red-orange* objects are autofluorescent tissue structures. Scale bar = 60 μm.

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THE EXPRESSION OF VIRAL ENVELOPE GENES can cause HIV-1-infected cells to fuse with CD4-receptor and coreceptor-expressing uninfected cells, leading to the formation of syncytia, or so-called multinucleated giant cells. Early histological studies have reported the presence of HIV-induced syncytia in tissues of infected individuals, and evidence for the involvement of both dendritic cells and lymphocytes in syncytia formation has been put forward.[1,2] The fusion of uninfected $CD4^+$ T cells has been suggested to contribute to their depletion, ultimately leading to a generalized immunodeficiency and increased susceptibility to AIDS-related illnesses. More recently, however, syncytia formation has generally been discounted as a major mechanism of T cell death because of their low frequency within infected tissues, and because of mounting evidence in favor of alternative mechanisms of T cell depletion, such as through persistent immune activation and abortive T cell infection. Thus, although the emergence of CXCR4-tropic, syncytia-inducing strains of HIV-1 in the circulation of infected individuals is associated with a more pronounced $CD4^+$ T cell decline and progression to AIDS, the pathophysiological significance of HIV-induced syncytia formation remains unclear.

We have recently begun to investigate the behavior of HIV virions and of HIV-infected immune cells *in vivo* through their visualization in the BLT humanized mouse model of HIV infection using fluorescent viral reporter strains and multiphoton intravital microscopy. Our initial studies, in which we explored the migratory behavior of T cells productively infected with CCR5-tropic, GFP-expressing HIV strains in the lymph node, revealed that the formation of T cell-derived syncytia occurred early (within 2 days) after initial infection. However, these were distinct from syncytia observed *in vitro* in that they were highly motile, and that individual nuclei moved in different directions, yet remained tethered to each other by fine membrane bridges.[3] Additionally, early syncytia, although stretching out over distances of more than 100 μm, typically contained no more than four to five discernible nuclei.

Here we report that only several days later, on days 5–6 after infection, some syncytia had dramatically increased in size, with volumes exceeding by 50-fold over those of individual, infected T cells (Fig. 1). They also adopted new morphological characteristics, such as a more massive cell body and an overall ''dendritic'' appearance, but nevertheless remained dynamic through the movements of their extensions. These changes may simply reflect their larger size compared to earlier syncytia, or could be the result of fusion with cells other than T cells to form mixed syncytia, similar to those observed in dendritic cells and T cell cocultures *in vitro*.[4]

We have shown that HIV-induced syncytia remain viable and motile in the lymph nodes of infected BLT mice. Using our intravital imaging approaches, we hope to address additional questions, including (1) how and from which cells they are formed, (2) how their formation is affected by changes in viral envelope fusogenicity, and (3) whether syncytia formation enhances viral replication and spread in some way, or is merely a viral ''accident'' and dead-end of viral replication.

Author Disclosure Statement

No competing financial interests exist.

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