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SPOTLIGHT

Virus update for the M2 "mac-in-touch"

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While HIV-1 infection of macrophages plays a major role in viral persistence and pathogenesis, how HIV-1 transfers from infected T cells to macrophages remains elusive. In this issue, Mascarau et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202205103) demonstrate how macrophage polarization drives their ability to fuse with HIV-1 infected T cells via the CD81/RhoA-ROCK/Myosin axis.

Current antiretroviral therapy (ART) has greatly increased the duration and quality of life for many people living with HIV-1. However, lifelong ART treatment is required and there is still no cure for HIV. This is because HIV can integrate into the host genome and persist in cellular reservoirs. These latent viral reservoirs contain transcriptionally inactive but replication-competent virus that can be reactivated upon discontinuation of ART, leading to major hurdles in complete eradication of HIV (1). As such, understanding cellular reservoirs and mechanisms of viral persistence are vital in achieving a cure for HIV.

$CD4^+$ T cells and cells of myeloid lineage such as macrophages and brain resident microglia are considered the main targets for HIV infection and contributors to viral persistence and pathogenesis (1, 2). While T cell reservoirs have been extensively studied, less attention has been paid to investigating myeloid reservoirs. Macrophages play a critical role in the establishment and persistence of the HIV reservoir owing to their long life span and their ability to reside in nearly every human tissue including immune privileged tissues such as the central nervous system. Moreover, HIV-infected macrophages are resistant to virally induced cytopathic effects, allowing viral replication for extended periods of time as opposed to infected T cells, which die within a few days of infection. Indeed, macrophages can assist in sustaining viral persistence in a humanized mice model that lacks $CD4^+$ T cells (3). Despite expressing viral entry receptors, although at a lower level than $CD4^+$ T cells, macrophages are poorly susceptible to infection by cell-free HIV-1, where viral particles bud off an infected cell before attaching to an uninfected cell. It has long been known that cell-to-cell transmission of HIV, where infected cells form virological synapses through close contact with an uninfected cell, is the predominant mode of infection in macrophages. However, the underlying mechanisms that control HIV dissemination by cell-to-cell transfer for infection of macrophages remain largely unknown.

In this study, Mascarau et al. address an important knowledge gap in our understanding of the mechanisms by which HIV-1 transfers from infected T cells to macrophages (4). They first evaluated the efficacy of the two currently known cell-to-cell transfer modes of macrophage infection in vitro; either by phagocytosis of the infected T cells or by heterotypic cell fusion with infected T cells. To do this, they analyze cell-to-cell transfer of HIV by coculturing primary human monocyte-derived macrophages (MDMs) and more importantly, tissue-resident macrophages isolated from various human tissues including alveolar and placental macrophages, with either Jurkat T cell line or primary $CD4^+$ T cells infected with HIV-1 in vitro. They showed that phagocytosis-mediated transfer happens mainly when HIV-infected T cells are apoptotic and is poorly efficient for infection of macrophages, and the most efficient HIV transfer occurs through heterotypic cell fusion between living infected T lymphocytes and macrophages, independently of their tissue localization (Fig. 1).

Macrophages exhibit great heterogeneity and can be driven toward pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes in response to local cytokine production (5). A polarization switch from M1 during acute phase of infection to M2 through late stages is believed to occur in vivo. Given that macrophage polarization is known to be critical for infection efficiency by cell-free HIV, the authors then evaluated the ability of the MDMs polarized into M1 and M2 phenotype to fuse with infected T cells in their coculturing system above. They showed that the synapse-like mode of infection is favored in pro-inflammatory conditions but results in low permissiveness of macrophages to infection, while heterotypic fusion is the main infection mechanism in M2-like MDMs. In both conditions, infection through phagocytosis remained minor. Their finding suggests that anti-inflammatory activation promotes infection of macrophages through heterotypic fusion with infected T cells.

Using a combination of live and fixed cell imaging, the authors further visualized the

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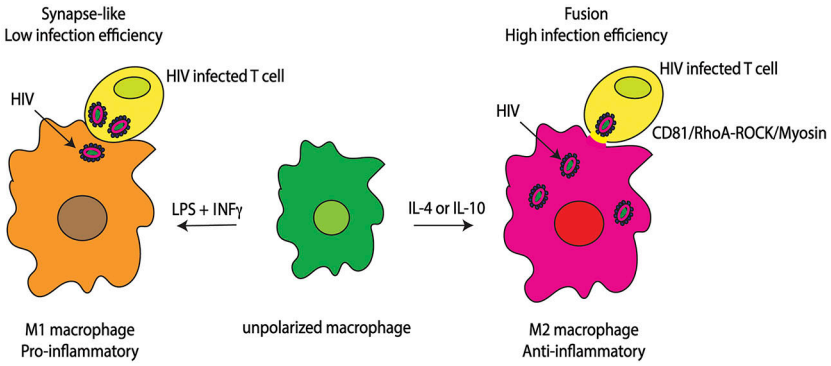

Figure 1. **Anti-inflammatory activation promotes infection of macrophages through heterotypic fusion with HIV-1–infected T cells.** Unpolarized macrophages can be driven toward pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes in response to local cytokine environment (lipopolysaccharide [LPS]-induced, IFN-γ, interleukin-4 [IL-4], or interleukin-10 [IL-10]), in different tissue compartments. M1 and M2 polarization affects the susceptibility of macrophages to HIV-1 infection. The synapse-like mode of infection is favored in pro-inflammatory conditions but results in low permissiveness of macrophages to infection, while heterotypic fusion with viable HIV-1–infected T cells is the main infection mechanism in M2 macrophages via the CD81/RhoA-ROCK/Myosin axis.

fusogenic contact of MDMs with T cells containing fluorescently tagged HIV-1 and showed that in contrast with uninfected T cells, MDMs cocultured with infected T cells became polarized, with an increased recruitment of CD18 tetraspanin and F-actin at cell contacts. Exploring this further, they demonstrated that either blocking CD18 by specific antibodies or using actin-disrupting drugs reduces the infection of MDMs by fusion with infected T cells. Further drug- or siRNA-mediated inhibition of myosin II confirms an inhibitory role of myosin IIA in MDM fusion with infected T cells, in line with known effect of actomyosin complexes in regulation of actin cytoskeleton. They also showed how CD81, another tetraspanin known to prevent fusion of macrophages (6), inhibits HIV-mediated heterotypic fusion. They found that upon macrophage–T cell contact, CD81 initiates a signaling cascade that activates RhoA/ROCK and subsequent phosphorylation of myosin IIA, which limits fusion of macrophages with infected T cells. Given that earlier studies mainly used transformed cell lines as virus producers and T cells as targets to show CD81-mediated inhibition of HIV (7, 8), these new findings described here contribute to a better understanding of the inhibitory role of tetraspanins in fusion processes.

Collectively, the work by Mascarau et al. provides important new insights into less well-understood aspects of HIV transmission by tissue-resident macrophages and the associated pathogenesis and establishment of persistent reservoirs (4). Their work could have implications in the understanding of HIV spread from infected T cells to other myeloid cells for which a similar heterotypic cell fusion mechanism has been recently described by themselves and others (9, 10). Indeed, cell-to-cell transmission of HIV-1 has recently been shown to overcome the entry block of non-macrophage-tropic virus strains in infecting macrophages, suggesting that HIV-1 might have a more prevalent tropism for macrophages than initially thought (11). These emerging studies are shedding light on HIV-1 cell-to-cell spread to macrophages in T cell–rich tissues and the subsequent establishment of viral reservoirs. While it will be challenging to determine the extent to which this fusion mechanism contributes to HIV-1 dissemination during infection in vivo, strategies to directly interfere with viral cell-to-cell spread and virus dissemination may provide a druggable target for reducing HIV reservoirs.

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