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REVIEWS

Review

On the Whereabouts of HIV-1 Cellular Entry and Its Fusion Ports

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HIV-1 disseminates to diverse tissues through different cell types and establishes long-lived reservoirs. The exact cellular compartment where fusion occurs differs depending on the cell type and mode of viral transmission. This implies that HIV-1 may modulate a number of common host cell factors in different cell types. In this review, we evaluate recent advances on the host cell factors that play an important role in HIV-1 entry and fusion. New insights from restriction factors inhibiting virus–cell fusion *in vitro* may contribute to the development of future therapeutic interventions. Collectively, novel findings underline the need for potent, host-directed therapies that disrupt the earliest stages of the virus life cycle and preclude the emergence of resistant viral variants.

HIV-1 Infects Different Cell Types

Tens of millions of people are infected with HIV-1 worldwide, an enveloped lentivirus of the Retroviridae family, and a similar number have died from the spectrum of AIDS-related diseases [1]. Despite the ability to administer **antiretroviral therapy** (**ART**; see Glossary) to prevent HIV-1 morbidity and mortality in patients with AIDS, this therapeutic approach does not eradicate the virus and remains inaccessible in many countries burdened with the majority of global infections [2]. Basic research focusing on the interaction of HIV-1 with numerous cell types is important to understand the mechanisms of virus persistence, transmission and pathogenesis. Existing ART treatment lowers viraemia to near undetectable levels but fails to eliminate HIV-1 from infected individuals, presumably due to the presence of **latent viral reservoirs** in $CD4^+$ T cells and other cell types (Boxes 1 and 2) [3]. Indeed, other cell types are susceptible to HIV-1 infection, such as **macrophages** and other hematopoietic cells of the myeloid lineage [4], and can also contribute to the establishment of chronic infection. While a well-established non-human primate model using simian immunodeficiency virus has revealed the importance of myeloid cell infection [5,6], a better understanding of how human tissue macrophages respond to HIV-1 infection is required to more effectively engineer strategies for viral eradication. The role of **dendritic cells** (DCs) in HIV-1 infection is clearly of interest, as they facilitate virus transmission to T cells despite being refractory to HIV-1 fusion and productive infection themselves [7]. It is possible that HIV-1 circumvents direct infection of DCs to prevent activation of an innate immune response. In this review, we consider recent advances in the study of HIV-1 entry in different cellular contexts. Indeed, the application of new imaging techniques (such as super-resolution microscopy [8], spectral imaging [9–11] or cryo-electron microscopy [12,13]) has led to recent discoveries highlighting the importance of the point of HIV-1 fusion in cells, and how HIV-1 can evade or antagonize host proteins that inhibit virus entry (also termed host **restriction factors**). Here, we describe important advances describing the

Trends

Recent studies of HIV-1 infection have described the role of dynamin-2 (DNM2) in stabilization of the fusion pore in the plasma membrane of $CD4^+$ T cells, and also shown that DNM2 in these cells might not be involved in HIV-1 virion endocytosis.

The role of endocytosis during cell–cell HIV-1 transmission is controversial: some reports state that HIV-1 might mature and fuse within endosomal compartments in the target cell, while others state that endocytosis might not lead to productive infection. In this scenario, the virus would be recycled back towards the plasma membrane and fuse. This is important because endosomal delivery of the virus could serve to evade neutralization by circulating HIV-1-specific antibodies.

Recent studies have highlighted the importance of the role played by host cells during HIV-1 entry. Indeed, inhibition of HIV-1 entry can be modulated by innate immunity, and by restriction factors such as IFITM and SERINC.

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molecular mechanisms leading to HIV-1 fusion in different cellular contexts, that is, macrophages, DCs and T cells, as well as the role of different host restriction factors during entry.

HIV-1 Entry into Macrophages

Endocytosis has surfaced as a potentially important route of HIV-1 entry [14,15]. Several studies have dealt with this hypothesis in different biological contexts. In particular, evidence in macrophages provides a strong case for virus–cell fusion occurring within endosomal compartments [10,16,17]. Macrophages exhibit high endocytic activity relative to T cells [18] and HIV-1 uptake following endocytosis could be a route to productive infection in cell lines [10]. In an initial study documenting the observation that HIV-1 could enter macrophages via endocytosis, Carter and co-workers [19] employed a pharmacological approach utilizing inhibitors of caveolin-1-related endocytosis, micropinocytosis, **actin** rearrangements, **actin remodelling**, and Rho GTPases, to determine that HIV-1 entry into macrophages proceeds, most likely, via an endocytic pathway related to micropinocytosis. Furthermore, genetic approaches have been used to demonstrate that the overexpression of CD4 at the surface of induced pluripotent stem cell (iPSC)-derived human macrophages (PSC macrophages) can increase HIV-1 fusion but not HIV-1 infection efficiency, as evidenced from **viral DNA expression assays** [20]. Indeed, HIV-1 was demonstrated to fuse within endosomes in iPSC-derived macrophages via GTPase **Dynamin-2** (DNM2), Ras-related C3 botulinum toxin substrate 1 (RAC1 GTPase) and Pak-dependent (kinase) pathway [21]. The authors measured fusion and **reverse transcription** in iPSC-derived macrophages expressing Lck tyrosine protein kinase, which tethered CD4 on the cell surface, altering its normal endocytic rate and increasing CD4 expression threefold. This led to a significant increase in HIV-1 fusion and reverse transcription but strikingly,

Box 1. HIV-1 Latent Reservoirs

Individuals infected with HIV-1 are now able to live longer than ever before, and their health remains stable as HIV-1 infection is kept under control by ART. However, HIV-1 infection remains a chronic illness due to the presence of latent viral reservoirs, which persist within cells. The nature of these reservoirs has been best characterized in $CD4^+$ T cells; however, the presence of viral reservoirs has also been observed in various other cells and tissues, such as in lymph nodes, gut-associated lymphoid tissue, spleen, liver and central nervous system [78]. HIV-1 is thought to accumulate in these reservoirs in the first few days of viraemia; however, several studies have shown that replication of HIV-1 within lymphoid tissues is ongoing, even during ART treatment [79]. A possible explanation for this observation has been attributed to unfavourable pharmacokinetics of commonly used antiretroviral drugs, which might only reach the lymph nodes at low concentrations [80]. In an attempt to eradicate these latent HIV-1 reservoirs, the so-called shock-and-kill strategy has been developed (Box 2).

A number of methods are being considered to address the problems associated with reactivating latent reservoirs, such as using improved **latency reversing agents (LRAs)**, neutralizing antibodies, **bispecific antibodies** and by engineering T cells using the **clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene editing** system [81]. Most LRAs target epigenetic factors; however, because viral reservoirs are present in a diverse range of cell types, the development of new small molecules or signalling molecules that act by altering the metabolism of cells may be required. Three to twelve months after the initiation of HIV-1 infection, broadly neutralizing antibodies (bNAbs) are produced by the host to target parts of the virus. The identification of successful bNAbs produced by patients is currently the subject of extensive and promising research. The hope is that bNAbs can be reproduced and combined to control viraemia during ART interruption, engaging the host immune system to fight HIV-1 [82,83]. It was recently shown that a bispecific antibody acting as a bNAb recognizing CD3 was able to increase the percentage of **follicular $CD8^+$ T cells**, which killed HIV-1-infected cells *in vitro* [84]. Another putative therapeutic approach using CRISPR/Cas9 has been used to eliminate the HIV-1 genome from human T lymphoid cells [85]. However, CRISPR/Cas9-derived mutations have been proposed to potentially accelerate viral escape [86,87].

A main obstacle to the eradication of HIV-1 remains the presence of latent viral reservoirs. These reservoirs are hidden inside a diverse range of cell types, not equally represented in the literature. To address this, further research is required to identify the relevant cell types, anatomic locations and the overall amount of latent viral DNA in HIV-1-infected patients.

Glossary

Actin: family of multifunctional proteins that form microfilaments. Actin participates in cell motility, cell division, cytokinesis, vesical and organelle movement, cell signalling and cell shape.
Actin nucleation: actin filaments are generated by actin polymerization. The first step is known as nucleation, involving the formation of three actin monomers from which filaments may elongate.
Actin remodelling: polymerization organized by actin monomers in response to signalling cascades initiated by environment cues. The remodelling of actin filaments is mediated by actin-binding proteins leading to changes in cell organization and shape.
ART: the gold standard in HIV-1 treatment. This usually involves the combination of three antiviral drugs, which act in different ways to limit HIV-1 spread. These may include fusion inhibitors, non-nucleoside reverse-transcriptase inhibitors, nucleoside reverse-transcriptase inhibitors, protease inhibitors and entry inhibitors or integrase strand transfer inhibitors. ART manages but does not cure HIV-1 infection.
BET family inhibitors: protein inhibitors that reversibly bind proteins of the bromodomain and extra terminal motif family and prevent protein–protein interactions between them and acetylated histones and transcription factors.
Bispecific antibodies: artificial protein composed of two fragments of two different monoclonal antibodies or a monoclonal antibody and a neutralizing antibody.
Bleb retraction: in mammalian cells, Blebs are spherical cellular prostrations, not supported by an actin cytoskeleton, observed during cytokinesis, apoptosis and cell migration. Retraction occurs when a cell membrane lacks the stability afforded by cell–substrate contacts.
CCR5: 7-transmembrane G protein-coupled chemokine receptor binding inflammatory chemokines. Upon binding, it transduces a signal that increases intracellular calcium. It acts as a coreceptor (CD4 is the primary) for HIV R5 strains.
CD4: membrane protein involved in the MHC–T cell interaction. It is the primary receptor for HIV-1.