PERSPECTIVE

Are the mechanisms involved in astrocyte and lymphocyte death during HIV infection similar?

Acquired immune deficiency syndrome is associated with the death of $CD4^+$ T lymphocytes. The entry of the human immunodeficiency virus (HIV) into the central nervous system leads to a broad spectrum of HIV-associated neurocognitive disorders (HAND) ranging from mild to severe dementia. Inside the central nervous system, HIV establishes infection in astrocytes – the predominant cell type in the brain, thus causing neuropathology, but the underlying mechanisms remain unknown. Much research has been focused on the role of innate immune activation, prompted by abundance of soluble viral factors, abortive infection, or cytokines secreted by neighboring microglia and associated with neuroinflammation and HAND. However, the mechanisms that prime and activate the inflammatory process during HIV infection have not been unraveled (Rawat et al., 2019).

Elucidating viral mechanisms involved in cell death: Studies involving immortalized T cell lines or activated cultures of peripheral blood cells infected with laboratory-adapted strains of HIV have shown that productive infection leads to cell death of virus-producing cells. However, considering the narrow rate of these activated $CD4^+$ T cells observed *in vivo*, the massive loss of $CD4^+$ T cells has involved not only the cytopathic effect but also an innocent bystander effect among non-actively infected $CD4^+$ T cells. Such deleterious outcome may imply an important role of both host (*e.g.*, tumor necrosis factor-α, Fas ligand, and TRAIL), and HIV proteins (*e.g.* Tat, Vpr, and Nef) released in their soluble forms or as content of exosomes from infected cells. Other permissive CD4-expressing cells such as monocyte-derived macrophages or microglia appear to survive for some months to years. Among them, viral replication is not inherently linked to cell death, and they are able to produce progeny over a period of weeks .

In vivo evidence has shown that 3–19% of astrocytes within the infected brain tissue carry HIV DNA (Churchill et al., 2009). Cell-free mature HIV does not efficiently infect astrocytes, but there are other routes of transmission: for example, cell-to-cell transmission of immature HIV virions between lymphocytes, or engulfment of HIV-infected macrophage materials have been linked to productive infection as well as functional changes during nonproductive infection of astrocytes that contribute to HAND cellular pathogenesis on astrocytes. These findings indicate that infection of astrocytes occurred efficiently by cell-to-cell contact with HIV-infected lymphocytes or monocytes and demonstrate a mechanism by which immature viral particles initiate a fusion process in a CXCR4-dependent and CD4-independent manner, thus promoting HIV transmission. It is possible that a trans-receptor mechanism may contribute to transmission among astrocytes, by which CXCR4 expressed on neighboring cells primes HIV envelope protein to fuse with a target cell that expresses appropriate co-receptors (Li et al., 2015; Russell et al., 2017) (**Figure 1**).

Such HIV-exposed astrocytes evidenced no cytopathic effect or bystander cell death according to the productive or abortive viral replication, respectively. When these two scenarios are juxtaposed in a cell-to-cell contact, the formation of gap junction channels promotes a contagious cell death by apoptosis involving several host (*e.g.*, reactive oxygen species, Ca^{2+} and inositol phosphate 3), and virus (*e.g.*, HIV-Tat, viral RNA) "toxic signals" shared between astrocytes (Malik et al., 2017).

Among $CD4^+$-T lymphocytes, the availability of primary lymphoid tissue using an *ex vivo* human lymphoid aggregate culture system highlighted a key role of bystander cell death. This system exhibited a near complete depletion of $CD4^+$ T cell population involving only 5% of productively infected cells but, conversely, 95% of the dying $CD4^+$ T cells were resting, non-permissive cells. Such a massive cell death of the resting $CD4^+$ T cells occurs among HIV abortively infected cells requiring cell-to-cell contact and involving cytosolic incomplete viral DNA chains generated during attenuated reverse transcription to promote bystander cell death by pyroptosis (Doitsh et al., 2014) (**Figure 1**).

Intrinsic antiviral innate immune response promoting cell suicide: Cells of the innate immune system use pattern recognition receptors (PRRs) to identify viral pathogens by engaging pathogen-associated molecular patterns (PAMPs). These PRRs are present either on the cell surface, within distinct intracellular compartments or in the cytosol, and include the Toll-like receptors, the retinoic acid-inducible gene I-like receptors, the nucleotide oligomerization domain-like receptors and cytosolic DNA sensors (Thompson et al., 2011). Astrocytes express a restricted Toll-like receptor repertoire that contributes to inflammatory responses mediating glial activation, cytokine production and neuronal damage during viral central nervous system infections.

HIV-induced quiescent lymphoid $CD4^+$ T cell death after accumulation of incomplete reverse transcripts involves DNA sensing by PRRs (*e.g.* IFI16, cGAS, STING) which triggers induction of a type-I interferon (IFN) response. Lastly, caspase-1-mediated inflammasome-dependent pyroptosis is promoted, a highly inflammatory form of programmed cell death which represents a common pathway of the host innate antiviral response even in the absence of active virus replication (Doitsh et al., 2014). The release of proinflammatory cellular contents, including ATP, by pyroptotic $CD4^+$ T cells may provide a second inflammatory stimulus, leading to activation of caspase-1 by the NLRP3 inflammasome in surrounding $CD4^+$ T cells, among other cells. Thus, HIV-mediated pyroptosis may trigger an avalanche of new rounds of pyroptosis in primed $CD4^+$ T cells by the repeated release of intracellular ATP in a virus-independent manner. Such an "auto-inflammation" scenario could generate persistent rounds of pyroptosis, chronic inflammation, and loss of $CD4^+$ T cells even when viral replication is reduced by antiretroviral therapy. However, blocking IFN I-mediated response among $CD4^+$ T cells did not prevent cell death thus indicating that this antiviral response is not essential for the innate immune-mediated onset of programmed cell death. This fact may be related to a switch between cell death modes as was described for influenza virus

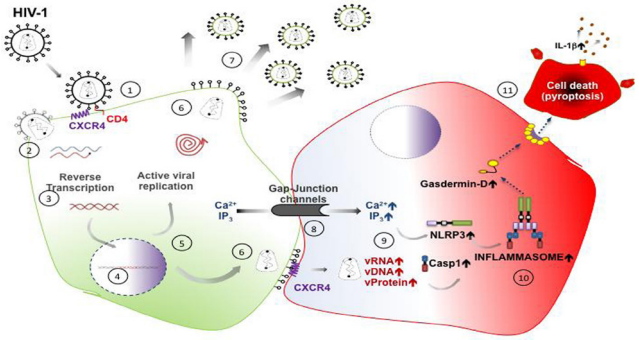

Figure 1 Overview of HIV-1 cell death mechanisms involved in both astrocytes and $CD4^+$ T lymphocytes.
Two cellular scenarios are distinguishable according to HIV replication outcome: productive HIV replication (green cell) and abortive HIV replication (red cell). HIV attachment is the first step on its replication cycle mediated by contact between viral envelope glycoproteins with cellular receptor ($CD4^+$)/coreceptors (CCR5/CXCR4). Next, viral envelope and cell membrane fuse (1), allowing the release of genomic RNA into the cell cytoplasm (2). Using reverse transcriptase, viral RNA is converted into DNA (3) which is the integrated into cellular genomic DNA in the nucleus (4). Once integrated into the cell DNA, HIV begins with transcription of its genes, de novo synthesis of genomes (RNA) and viral protein expression (5). These synthetized components are assembled into immature (non-infectious) viral particles, with expression of viral envelope glycoproteins on the cell membrane (6). These immature HIV particles are released by budding from the cell membrane. Finally, HIV maturation mediated by viral protease activity on long viral proteins occurs at extracellular sites (7). During viral replication, intracellular concentration of Ca^{2+} and inositol phosphate (IP_3) increases and Ca^{2+} then diffuses through gap junctions to adjacent cells (8). Cell-to-cell fusion (by viral glycoproteins-coreceptor contact) facilitates the invasion of neighboring cells by immature particles and/or viral components (9). A cascade of events including mitochondria-derived oxidative stress, inflammasome activation (NLRP3-Caspase-1-GSDMD pathway) and bystander cell death by pyroptosis (10) and IL-1β release (11) may occur during abortive-infection. HIV: Human immunodeficiency virus; IL-1β: interleukin 1 beta.

among epithelial cells where apoptosis is induced at early phases of infection, but the cell death pathway is shifted to pyroptosis at late phases of infection mediated by the type I IFN mediated Janus kinase-signal transducer and antivator of transcription signaling pathway (Lee et al., 2018).

A striking difference is observed in the biology of resting $CD4^+$ T cells residing in lymphoid tissue versus blood. Although both tissues support HIV entry and fusion with equivalent efficiency, blood cells were highly resistant to the pyroptotic death pathway considering their deeper state of cellular rest. This state is associated with the formation of fewer incomplete reverse transcripts following abortive infection and lower expression of innate immune sensors that do not lead to the expression of IFNβ- and IFN-stimulated genes. Consequently, the pyroptosis actors that are constitutively expressed in lymphoid tissue $CD4^+$ T cells are not expressed among resting blood-derived $CD4^+$ T cells (Berg et al., 2014). Innate immune mediators and the microenvironment within lymphoid tissues are evidently crucial in the massive bystander killing of $CD4^+$ T cells that occurs during HIV infection.

Foreign DNA sensing by PRRs such as IFI-16 may also occur among astrocytes but their involvement for the depletion of bystander cell death following abortive HIV infection is still unknown. In line with this, using normal human astrocytes we recently demonstrated such bystander cell death as infection outcome among non-productively infected cells. Among these cells, under evident mitochondrial dysfunction, both damage- and pathogen-associated molecular patterns (DAMPs and PAMPs, respectively) trigger inflammasome activation *via* NLRs, thus promoting caspase-1 and Gasdermin D cleavage which ultimately result in release of mature interleukin-1β. Furthermore, inflammasome activation was almost totally abolished after caspase-1 inhibition, or using a mitochondrial reactive oxygen specie scavenger. In contrast, HIV-productively infected astrocytes were able to evade such innate immune response by maintaining the mitochondrial mass healthy despite the substantial energy demand. An exacerbated selective autophagy of mitochondria ('mitophagy') is observed among productively infected cells, thus preventing the accumulation of damaged mitochondria that can lead to inflammasome activation (Ojeda et al., 2018) (**Figure 1**).

Cell-to-cell transmission of HIV triggering cell death: Retroviruses fuse and enter their target cells either as cell-free virions or through cell-to-cell spread that is 102 to 103 times more efficient than spreading of cell-free particles through virological synapses stabilized through actin-mediated recruitment of adhesion molecules (lymphocyte function antigen-1 and its cognate ligand, intercellular adhesion molecule1). Paradoxically, although this phenomenon is efficient for viral dissemination among permissive cells, it acts against HIV when it spreads to non-permissive targets such as more prevalent resting non-permissive $CD4^+$ T cells, causing termination of viral propagation, abortive infection, and cell death by caspase-1-dependent pyroptosis driving inflammation and disease (Galloway et al., 2015). Conditioned medium from lymphoid tissues fails to consistently render peripheral blood cells sensitive to cell death by pyroptosis, suggesting that key signals are generated through cell-to-cell interactions.

As in lymphoid tissues, immune mediators, host and viral factor dissemination within the brain parenchyma may promote the massive bystander cytopathic effect on astrocytes. However, unlike what happens with $CD4^+$ T cells, astrocyte activation through the binding of viral soluble proteins (*e.g.* Tat) or cytokines secreted by neighboring infected astrocytes or microglia, could drive the expression of several toll-like receptors that can bind DAMPs and PAMPs (Serramia et al., 2015). Otherwise, virological synapses involving infected $CD4^+$ T cell and uninfected astrocytes has been shown to likely promote the transfer of large amounts of immature virus. Such contagious transmission of immature viral particles may provide a second inflammatory stimulus, controlling the activation of caspase-1 by the NLRP3 inflammasome in neighboring primed uninfected astrocytes. Therefore, astrocyte death may occur by released factors and through cell-to-cell interactions (**Figure 1**).

In conclusion, neurocognitive complications, typically known as HAND, still persist in the era of effective antiretroviral medications. Our current understanding of the similarities and dissimilarities about HIV pathogenesis in astrocytes and lymphocytes during infection claims further research to clarify whether death signals that act on the abortively HIV-infected $CD4^+$ T lymphocytes are the same as those involved in astrocytes. To shed light on this, in-depth analyses using selective inhibitors of the different stages of HIV replication will be crucial to highlight the role of each of the viral PAMPs in the activation of the inflammasome.

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