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# A Continuing Journey for HTLV-1 Antiviral Development?

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The study of the path followed by a virus in a host cell may be of great interest for therapeutics. Indeed, it is quite possible to imagine that any modulation made to this path can affect the cycle of this virus and therefore its transmission [1].

Starting from this paradigm, we focused, in the work [2] we published in 2017, on the study of the traffic of the retrovirus HTLV-1 (Human T cell leukemia/lymphoma virus type 1) in several dendritic cells (DCs) subsets. DCs are professional antigen-presenting cells located in the skin, mucosa and lymphoid tissues. Their function is to capture, process and then present antigens to mount a specific adaptive immune response. The retrovirus HTLV-1 is the etiological agent of Adult T cell Leukemia/Lymphoma (ATLL), a lymphoproliferative disease and HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP), a neurodegenerative disease (for review [3]). This virus is thought to first infect DCs *in vivo* [4]. HTLV-1 then spreads to the T cells that constitute the predominantly infected cell population in chronically infected individuals.

The development of ATLL has been associated to an HTLV-1 entry through the digestive tract [5], after long-term breast-feeding, while the development of HAM/TSP has been associated to an HTLV-1 entry by blood or after unprotected sexual intercourse [6], all these interfaces being colonized by several subsets of DCs. We thus wondered how HTLV-1 interacts with those various subsets of dendritic cells and whether these interactions would lead to different viral path, possibly linked to different disease outcomes. To test our hypothesis, we used *in vitro* monocyte-derived DCs cultured using different cytokine cocktails in order to generate different DCs subsets: the IL-4 DCs and the IFN-α DCs which mimic the myeloid DCs of the blood and the inflammatory DCs of the mucosa respectively. First, using selective inhibition of various endocytosis pathways, we tested whether HTLV-1's traffic was similar in both DC subsets and, then, whether these two DCs subsets get productively infected by HTLV-1. We finally assessed whether the nature of the DCs subset would have an impact on the spread of HTLV-1 to T-cells.

We first showed that HTLV-1 enters the IL-4 DCs using clathrin-mediated endocytosis and macropinocytosis while it only uses macropinocytosis in the IFN-α DCs. Nonetheless, HTLV-1 particles are localized in CD82+ vesicles in both IL-4 DCs and in IFN-α DCs, suggesting that although HTLV-1 uses different entry pathways, those pathways converge to similar vesicles in which HTLV-1 is accumulated in the two DCs subsets. Moreover, the physical properties of these vesicles in the two subsets are different; those of IL-4 DC have a neutral pH, while those of IFN-α DCs are acidic. We showed that these physical differences of the two DCs subset's vesicles impact the faith of the infection and then the ability of the DCs to transmit infection to T-cells. Indeed, the traffic of HTLV-1 into the acidic vesicles of IFN-α DCs restricts both productive infection and viral transmission to T-cells whereas its traffic through the neutral vesicles of IL-4 DCs authorizes their productive infection and also their ability to transmit HTLV-1 to T-cells.

By considering these different results which oppose the two subsets of DCs, we propose that the acidification of the intracellular compartments of the DCs could constitute a future therapy for limiting the transmission of HTLV-1. The intracellular pH of the vesicles or

endosomes depends on the coordinated activity of two enzymes: NADPH oxidase (NOX2) and V-ATPase (Vacuolar-type $H^+$-ATPase) [7]. Since DCs express NOX2 [8], they produce ROS (reactive oxygen species). The continuous ROS production, combined with a low V-ATPase activity in IL-4 DCs [9], results in the consumption of protons leading to the alkalization of vesicles [9,10]. The subcellular assembly of NOX2 to vesicles is controlled by Rac2, a small signaling G protein and in its absence, the production of ROS by the endosomes of DCs is abolished [11] and the pH of these endosomes is thus acidic. Rac2 down regulation in patients may be difficult to reach. However, direct inhibition of the NOX enzymes could be a more accessible strategy. For example, DPI (for diphenyleneiodonium chloride), that targets NOX enzymes, allows the rapid acidification of endocytosis compartments [12], and in our experiments, statistically decreased the ability of HTLV- 1 to productively infect IL4-DCs [2]. In this context, it would be of great interest to test whether the treatment of infected patients with the inotropic agent DPI-201106 (used nowadays to selectively reverse the multidrug resistance in cancer cell lines [13]) could constitute a therapeutic strategy to eradicate HTLV-1. Furthermore, it is also possible to test several molecules that act on the V-ATP pathways and determine whether and how they modulate the course of HTLV-1 transmission from DCs to T cells. In this context, it is interesting to test two increasingly known V-ATPase inhibitors in therapeutics: the archazolid which is actually used to reduce cancer metastasis [14] and the concanamycin A that inhibits the androgen receptor expression in prostate cancer cells [15].

Additionally, HTLV-1 traffic to either acidic compartments in IFN-α DCs or to neutral compartments in IL-4 DCs may reflect HTLV-1 interactions with DCs receptors, in particular the lectin DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin), and as such increases the putative therapeutic intervention. This receptor is known to facilitate the capture of many viruses by the DCs. This is the case of the dengue virus [16], HIV-1 [17], Ebola [18], Marburg and Coronavirus [19]. Two DC-SIGN motifs, the LL motif (a dileucine) and the EEE cluster [20], are involved in the intracellular trafficking of the DC-SIGN/virus complex, and the mutation of the LL motif prevents the capture and endocytosis of viruses like phleboviruses [21]. Since IL-4 DCs express DC- SIGN and IFN-α don't, it is legitimate to link the interaction with, and maybe the signalization through, DC- SIGN to the ability of HTLV-1 to productively infect DC and to their viral transmission occurrence. It is also legitimate to ask whether neutralization of DC-SIGN using antibodies could be a therapeutic strategy for limiting the transmission of HTLV-1. This hypothesis is supported by several articles.

First, the reference to the work of El Awady *et al*. on *Porphyromonas gingivalis* is quite evocative of what we observed in our two subsets of DCs [22]. Indeed, El Awady *et al*. showed that when *Porphyromonas gingivalis* is captured via DC-SIGN (which may be the case for HTLV-1 capture by IL-4 DCs), it concentrates outside the intercellular compartments of autophagy, a cellular machinery that allows the orderly degradation and recycling of cellular components and infectious agents, thus preventing *P. gingivalis* degradation and allowing *P. gingivalis* persistence in DC. In contrast, when the capture of *P. gingivalis* is independent of DC-SIGN (which may be the case for HTLV-1 capture by IFN-α DCs), *P. gingivalis* is internalized in early phagosomes and then in phagophores, two structures involved in the degradation of the infectious particle. Similarly, Ribeiro *et al*. showed that the inhibition of autophagy through the extinction of Atg5 or Atg16L1 (two genes that control autophagy) resulted in an increase of HIV-1 integration in DCs and of their productive infection [23]. These results are reminiscent of ours in which the use of chloroquine, an inhibitor of the autophagy that also neutralizes the acidic endosomes, restored HTLV-1 productive infection in IFN-α DCs [2].

Second, the expression of DC-SIGN correlates with the inhibition of cellular antiviral responses that probably allow retroviruses to productively infect DCs. Indeed, DC-SIGN engagement recruits and activates the Raf-1 kinase that in turns inhibits the activity of PP1 phosphatases (for protein serine/threonine phosphatase) that are involved in various signaling pathways [24]. Inhibition of the PP1 phosphatases results in a decreased activation of RIG-I-like receptors (RIG-I) which are cytosolic detectors of mRNA [25] and activator of the innate type-I interferon antiviral response. It is through this mechanism that DC-SIGN contributes, for example, to increase the replication of the measles virus [26]. In contrast, neutralization of DC-SIGN before the exposure of myeloid DCs or plasmacytoid DCs, two *in vivo* DCs subsets, to respiratory syncytial virus leads to increased maturation and cytokine production by these two subsets of DCs (IFN-α, IL-6, IL-8, MIP-1α, MIP-1β) [27] favoring thus the control of the viral infection. Thus, interaction with DC-SIGN could be one of the mechanisms permitting the productive infection of DCs by many viruses: HTLV-1 [28], influenza A [29], dengue [16], HIV-1 [17].

Finally, the DCs in which DC- SIGN is engaged (as IL - 4 DCs in our study) could constitute a protective niche for the persistence of pathogens through potential mechanisms mediated by DC-SIGN: an escape from innate immunity induction together with an escape from degradation through autophagy or viral replication inhibition by restriction factors, all these mechanisms resulting in productive infection and viral transmission.

In conclusion, the acidity of the compartments in which HTLV-1 concentrates as well as the traffic towards compartments of autophagy may be two factors that limit the viral transmission of HTLV-1. Our work that shows that HTLV-1 productive infection of IL4-DCs relies on its traffic to neutral compartments distinct from autophagy pathway provides evidence that alternative therapies (such as inhibition of autophagy) could be considered to limit the transmission of HTLV-1. Such therapies, are nowadays tested in oncology [30,31]. Furthermore, development of DC-SIGN inhibitors is a promising alternative as it could transform the field of HIV, *Herpes* and HTLV-1 prevention by improving the efficacy of currently available antiviral treatments [32]. Such targeted therapies would finally circumvent the undesirable effects that are inherent to current antiviral treatments of HTLV-1 [33] and could contribute to prolonged overall survival.

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