HIV-1 virion accumulation and ATP-Induced release in human primary monocyte-derived macrophages

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Background: Macrophages are targets of HIV-1 infection and represent viral reservoirs in individuals receiving cART. A peculiarity of macrophage infection is their capacity to store new progeny virions in intracellular vacuolar compartments of debated origin. Moreover, functional polarization of macrophages towards the pro-inflammatory M1 or anti-inflammatory M2 cells restricts HIV-1 replication by different mechanisms (E. Cassol et al., J. Immunol. 2009). Therefore, we investigated whether human primary monocyte-derived macrophage (MDM) polarization involved the accumulation and release of HIV-1 virions upon acute infection and stimulation with extracellular ATP.

Methods: Human MDM were obtained from purified monocytes of HIV seronegative donors and were polarized (or not) into M1- or M2a-MDM as published (ibidem) and infected either with an R5 HIV-1 strain or with a VSVg-pseudotyped vector expressing eGFP. The cells were then stimulated or not with ATP (known to induce IL-1b release from intracellular compartments) in order to induce the potential release of virions from intracellular vesicles. In addition, the U937-derived promonocytic cell line U1, chronically infected with HIV-1, was differentiated to macrophage-like cells by PMA and was then stimulated with urokinase-type plasminogen activator (uPA) to favor retention of virions (M. Alfano et al. Blood, 2009) and then exposed to ATP to induce their release. Virion production was evaluated by the RT activity assay; other techniques were applied when appropriate.

Results: Extracellular ATP induced HIV virion release from both unpolarized and polarized HIV-infected MDM and in PMA-differentiated U1 cells stimulated with uPA. Virion release in MDM was associated with a reduction of intracellular virion retention, as measured by intracellular p24 staining and FACS analysis. We are currently analyzing virion morphogenesis and subcellular localization in polarized vs. unpolarized MDM and we are exploring additional pathway stimulating or preventing virion release in these cells.

Conclusion: ATP stimulation led to induction of preformed virion release in both primary MDM and in PMA-differentiated U1 cells stimulated with uPA. The identification of potentially “druggable” targets leading to either retention or release of preformed virion release by macrophages could be of significant relevance for identifying novel strategies of purging HIV-1 reservoirs in individuals under cART.