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Induction of G2 Cell Cycle Arrest in HIV-1 Infected Patients Mediated by HIV-1 Viral Protein R and the Regulation of HIV-1 Replication in Macrophages Mediated by Viral Protein R

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HIV infection leads to numerous cellular responses. So far it has not been possible to specifically identify the productively infected target lymphocyte in the peripheral blood of HIV-infected patients to allow for their independent characterization. We employed an intracellular p24 immunostaining assay in conjugation with flow cytometric analysis to identify productively infected lymphocytes. We applied this system to in vitro cultures and PBMC isolated from recent HIV seroconverters. In vitro infections with the wild-type and isogenic ΔVpr HIV-1 molecular clones allowed for the identification of productively infected lymphocytes that displayed a G2/M cell cycle arrest in a Vpr-dependent manner. Intracellular $p24^{Gag}$ staining of PBMC from 9 patients showed a variable number of HIV-infected lymphocytes that did not correlate with viral load. DNA content studies of these

infected lymphocytes revealed arrest at the G2/M phase of the cell cycle when compared with uninfected cells from these same patients.

These findings demonstrate that intracellular anti-HIV $p24^{Gag}$ staining can be employed to identify productively infected lymphocytes that display a G2 cell cycle arrest phenotype that is seen both in vitro and in PBMC isolated directly from the peripheral blood of recent seroconverters.

We further investigated the role of Vpr as a nucleocytoplasmic shuttling protein. HIV-1 and other lentiviruses are distinguished from the oncoretrovirus by their ability to infect non-dividing cells such as macrophages, which form an important viral reservoir within the infected host. Rather than requiring nuclear membrane breakdown during cell division, the PIC of HIV is able to localize to the nucleus by traversing the central aqueous channel of the nuclear core complex. The viral PIC contains three nucleophilic proteins including matrix, integrase and Vpr that are thought to collaborate on nuclear targeting of that complex. The mechanism by which Vpr can unfold such nucleophilic properties and yet be incorporated into nascent virions assembling at the plasma membrane remains unclear. In our laboratory, we have recently characterized Vpr as a nucleocytoplasmic shuttling protein containing two novel nuclear import signals and an exportin-1-dependent nuclear export signal. We find that mutation of the NES of Vpr impairs its incorporation into new virions. We further demonstrate that normal function of the Vpr NES is critical for efficient HIV replication in macrophages investigated in ex vivo lymphoid histocultures of human tonsil and spleen. We thus demonstrate how the nucleocytoplasmic shuttling property of Vpr not only leads to efficient PIC import, but also allows for the presence of Vpr in the cytoplasm, enforcing incorporation into newly formed virions and subsequently augmenting HIV replication in non-dividing macrophages.