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Nef inhibition for enhanced NK cell killing of cells expressing reactivated HIV-1

Scully E.1,2, Lockhart A.1, Garcia Beltran W.1, Bodair A.1, Rouyez M.-C.3, Benichou S.3, Kuritzkes D.2, Altfeld M.4
1Ragon Institute of MGH, MIT and Harvard, Cambridge, United States, 2Brigham and Women's Hospital, Division of Infectious Disease, Boston, United States, 3Institut Cochin, Paris, France, 4Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany

Background: Functional cure of HIV-1 infection obligates near complete eradication of cells carrying latent provirus. Early studies suggest that endogenous immune responses are insufficient and new strategies are needed to enhance immune recognition. The HIV-1 Nef protein mediates immune evasion by downregulating surface expression of CD4, HLA class I, and NKG2D receptor ligands. NKG2D is a receptor expressed on several classes of lymphocytes, and is a potent trigger of cytotoxicity on NK cells. We investigated the potential for inhibition of Nef to enhance NK cell killing of cells harboring latent HIV-1 after reactivation.

Methods: The J89 T cell line containing an integrated copy of HIV-1 with an eGFP reporter was used as a model of latency. HIV-1 expression was induced by treatment with 20 nM of panobinostat. Nef inhibitors included (1) a single-domain antibody fragment (sdAb19) previously shown to inhibit Nef-induced CD4 downregulation (2) a fusion of this antibody fragment to the SH3 domain of Hck, (Neffin), that blocks both CD4 and HLA class I downregulation. The sdAb19 and Neffin were stably expressed in the J89 cell line through transduction of lentiviral constructs. Their inhibitory activity was tested on Nef-induced downregulation of CD4, HLA class I, and NKG2D ligands. The killing activity of NK cells purified from HIV infected individuals was also assessed by coculture with varying ratios of J89 cells expressing the Neffin.

Results: In J89 cells, panobinostat induced expression of HIV-1 as measured by both GFP expression and intracellular p24. Concurrent expression of the sdAb inhibited Nef-mediated CD4 downregulation in reactivated cells, while expression of Neffin inhibited both CD4 and HLA class I downregulation. Both sdAb and Neffin blocked NKG2D ligand downregulation in reactivated J89 cells. There was a significant decline in the ratio of HIV+Neffin+:HIV+Neffin- in the NK cell killing assay. Mean ratio HIV+Neffin+:HIV+Neffin- was: 2.975 for no NK cells, 2.396 for 5:1 NK:target, and 1.087 for 10:1 NK:target (p=0.025 paired t test between no NK and 10:1).

Conclusions: These data indicate that Nef inhibition can enhance expression of NKG2D ligands after reactivation of HIV-1. The presence of Nef inhibitors enhances NK cell-mediated killing of cells expressing HIV-1 after reactivation.