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Characterization of cell surface downregulation of natural killer cell ligands by patient-derived human immunodeficiency virus type 1 Vpu and Nef alleles

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Natural killer (NK) cells constitute an essential first line of defense against malignancies and viral infections. Increasing evidence suggests that NK cells play a critical role in the immune response to HIV-1 infection. Consequently, HIV-1 has evolved several strategies to evade NK cell recognition that mostly rely on the viral accessory proteins Vpu and Nef. Vpu and Nef are known to downregulate the HIV-1 entry receptor CD4 and to enhance viral release and virion infectivity. In addition to these well-established activities, Vpu and Nef were recently shown to downregulate NK cell activating ligands, thus interfering with NK cell recognition of infected cells. While the modulation of NK cell activity by Vpu and Nef has been demonstrated in vitro, the in vivo relevance remains unknown. Thus, a comprehensive characterization of patient-derived Vpu and Nef alleles focused on the modulation of NK cell ligands was performed. The analysis aimed at evaluating the degree to which NK cell modulating activities are conserved among natural Vpu and Nef variants.

To this end, vpu and nef alleles were amplified from peripheral blood mononuclear cells (PBMCs) of HIV infected patients. Up to ten vpu or nef sequences were obtained for each patient and a representative clone selected for functional analysis. 17 Vpu and 20 Nef variants were compared in their ability to downregulate CD4, HLA-I, tetherin, and the NK cell activating ligands PVR, MICA, ULBP-2,5,6, and NTB-A. Consistent with previous studies, CD4, HLA-I, and tetherin downregulation activities were highly maintained across all alleles analyzed. Similarly, Nef alleles displayed a pronounced downregulation of the NK cell activating ligand PVR whereas this activity was less conserved among Vpu variants. Within the cell system used for functional analysis, only weak effects of Vpu and Nef on the expression of NTB-A and MICA were observed. In contrast, the NK cell activating receptor ULBP-2,5,6 was shown to be downregulated not only by Nef as previously described, but also by Vpu variants.

In a sideline of this thesis, natural occurring polymorphisms in Vpu and Nef associated with HIV-1 adaptation to NK cells were investigated. Specific amino acid changes in HIV-1

reportedly enhance binding of inhibitory NK cell receptors to HIV-1 infected cells, leading to viral escape from NK cell-mediated killing. One hypothesis to explain how NK cells impact HIV-1 evolution is that NK cell associated polymorphisms in HIV affect the ability of Vpu and Nef to downregulate NK cell activating ligands. In order to test this hypothesis, Vpu 71R/74L and Nef S9K mutants were generated and characterized in their ability to downregulate CD4, HLA-I, tetherin, and NK cell activating ligands. NK cell associated polymorphisms did not affect the ability of Vpu and Nef to modulate the expression profile of NK cell activating ligands. Furthermore, Vpu and Nef were not impaired in their ability to enhance viral release and virion infectivity, respectively. These findings support the alternative hypothesis that polymorphisms in Vpu and Nef occur in regions presented as HLA-C bound peptides to NK cells. Amino acid variations in viral peptide sequences might increase the binding affinity of inhibitory NK cell receptors to infected cells and confer resistance to NK cell-mediated lysis. Current studies that investigate the impact of peptide sequence variations on the binding of NK cells to HLA-C bound peptides will provide further insights into the mechanisms underlying the protective effect of NK cell-associated HIV-1 polymorphisms.