ABSTRACT

There is an increase in cardiovascular disease in people living with HIV (PLWH). Literature suggests the observed increased risk in this population is due to the HIV-1 Nef protein which causes endothelial dysfunction. Nef is a polymorphic accessory protein of which mutations in Nef have been associated with disease outcome. In a previous study, H40Y was associated with increased plasma concentration of markers of endothelial activation. This project therefore sought to confirm the reported effects of the H40Y polymorphism on endothelial cells. HIV-Nef expressing clones were constructed using an HIV-1 *nef* gene isolated from a clinical sample and the pMJ4 plasmid. The Nef-40Y variant was generated by site-directed mutagenesis. Human pulmonary microvascular endothelial cells (HPMECs) were transfected with the Nef-expressing constructs to determine the expression of the adhesion molecules ICAM-1 and VCAM-1 using RT-qPCR and ELISA analyses. The HIV-1 Nef-40H and Nef-MJ4 variants significantly increased ICAM-1 and VCAM-1 mRNA expression compared to HIV-1 Nef-40Y. Additionally, HIV-1 Nef-40H and Nef-MJ4 were seen to work in synergy with TNF-α to significantly increase VCAM-1 mRNA expression. However, a multiple comparisons test revealed that no variant significantly outperformed the other when measuring the concentration of soluble VCAM-1 proteins. No synergistic relationship was observed between the HIV-1 Nef variants and TNF-α at the protein level. This suggests a weak correlation between VCAM-1 mRNA and protein expression following Nef-induced endothelial activation. This study demonstrated that the HIV-1 Nef-40H variant had a greater negative effect on endothelial function compared to the Nef-40Y variant.