Live Cell Imaging of ABCA1 Downregulation by HIV-1 Nef in an Experimental Model of HeLa ABCA1-GFP

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FIG. 1. Effect of Nef expression on ABCA1 localization. HeLa ABCA1-GFP cells were transfected with a vector expressing HIV-1_{SF2} Nef-RFP and live cells were observed by fluorescent microscopy. The *upper panel* shows a composite image of two channels representing ABCA1-GFP (*green*) and HIV-1_{SF2} Nef-mRFP (*red*), whereas *panels* in the *bottom* represent single channels. *Letters* and *arrows* in the *bottom panels* identify cells not expressing Nef (A and B), cell with low expression of Nef (C), and cell with high Nef expression (D). Bar size is 20 μ m. Color images available online at www.liebertpub.com/aid

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EXPERIMENTAL MODEL OF HeLa ABCA1-GFP was developed by Neufeld *et al.*¹ to study intracellular localization and trafficking of ABCA1, the ATP-binding cassette protein that is involved in apoA-I-mediated cholesterol efflux, and formation of high-density lipoprotein. Our laboratory adopted this model for investigation of the role that HIV-1 plays in the pathogenesis of atherosclerosis in HIV-infected subjects. Our previous study points to HIV-1 Nef as the major factor responsible for the disruption of cholesterol efflux from infected cells by impairing the interaction between the cholesterol transporter ABCA1 and calnexin.² As a consequence, ABCA1 is retained in the endoplasmic reticulum and eventually undergoes degradation. The continuous overexpression of HIV-1 Nef can lead to almost undetectable levels of ABCA1. To observe the dynamics of Nef-induced changes in ABCA1 abundance and localization, we transfected HeLa ABCA1-GFP cells with plasmid expressing HIV-1_{SF2} Nef-mRFP (kind gift of Dr. Fackler) and imaged live cells over a period of 48 h. A snapshot captured at 26 h post-transfection is presented in Figure 1. The presented image was captured with an Axio Cell Observer Spinning Disk fluorescent microscope (Carl Zeiss) equipped with Yokogawa CSU X1 spinning disk and Evolve Delta EM CCD cameras (512 × 512, Photometrics), using a Plan Apochromat 100×/1.46 oil immersion lens. The image was processed using Volocity software (PerkinElmer Life Sciences). Cells with no detectable expression of HIV-1_{SF2} Nef-mRFP (Nef) (marked A and B in Fig. 1) have a prominent presence of ABCA1-GFP throughout the cell and on the plasma membrane, whereas the cell exhibiting high expression of Nef

(cell marked D) has only barely detectable ABCA1. Interestingly, in the cell with low Nef expression (cell marked C), ABCA1 seems to be localized primarily intracellularly, with very low presence on the plasma membrane.

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Author Disclosure Statement

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