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HIV-1 Infection of Macrophages Induces Retention of Cholesterol Transporter ABCA1 in the Endoplasmic Reticulum

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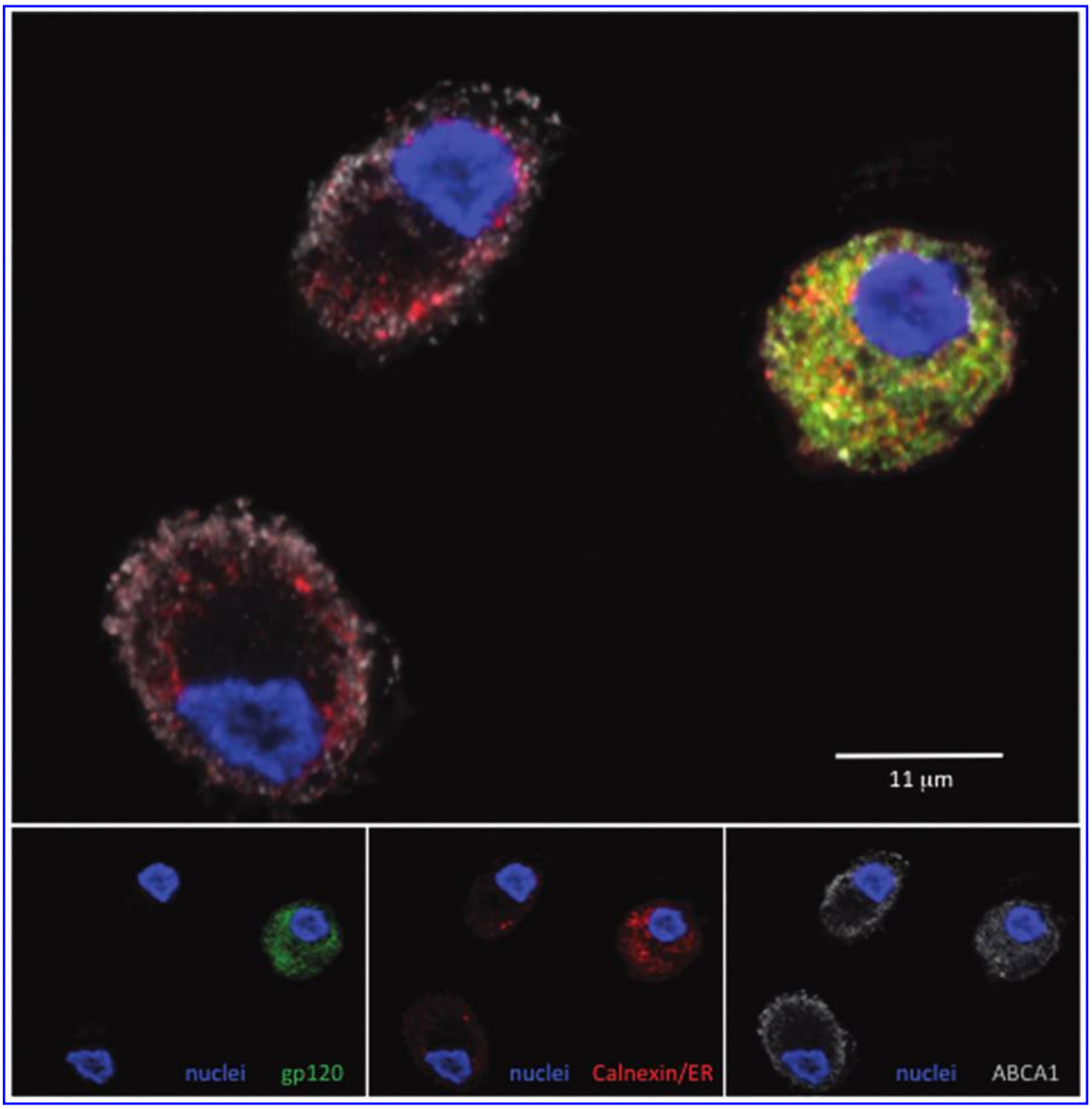

FIG. 1. ABCA1 localization in one gp120-positive and two gp120-negative cells of HIV-infected macrophage culture. The *upper panel* shows a composite image of four channels representing gp120 (*green*), calnexin (*red*), ABCA1 (*white*), and nuclei (*blue*), whereas panels in the *bottom* show combinations of two selected channels.

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HIV-1 INFECTION IS ASSOCIATED WITH a high risk of developing atherosclerosis,[1] and studies in animal models and *in vitro* demonstrated that impairment of activity of the cholesterol transporter ATP-Binding Cassette A1 (ABCA1) is a significant contributor to this side effect of HIV infection.[2,3] Our unpublished observations demonstrated that Nef binds to endoplasmic reticulum (ER) chaperone calnexin. While this binding does not affect the interaction between calnexin and gp160, it disrupts the interaction of calnexin and ABCA1. Given that calnexin is essential for the folding and maturation of ABCA1, this effect of Nef may explain Nef-mediated impairment of ABCA1 activity. Here, we provide images that demonstrate the effects of HIV infection on ABCA1 localization in macrophages.

Macrophages are the key cells in the development of atherosclerosis, and HIV-mediated disruption of ABCA1 activity in these cells contributes to cholesterol accumulation and their transformation into ''foam'' cells, the signature cells in atherosclerosis.[2] To investigate the mechanisms of ABCA1 inactivation, we infected monocyte-derived macrophages with VSV-G-pseudotyped HIV-1 and stained for HIV-1 Env (using anti-HIV gp120 sheep serum as a primary antibody and Alexa Fluor 488-conjugated donkey antisheep IgG as a secondary antibody), ABCA1 (using anti-ABCA1 rabbit polyclonal antibody as a primary antibody and Alexa Fluor 647-conjugated goat antirabbit IgG as a secondary antibody), and ER chaperone calnexin (using mouse monoclonal anticalnexin-ER membrane marker antibody as a primary antibody and goat antimouse DyLight 550-conjugated antibody as a secondary antibody), which regulates the folding and maturation of glycosylated proteins, including ABCA1 and gp160, in the ER. Nuclei were counterstained with DAPI. Images were captured with a Carl Zeiss LSM 710 confocal microscope, using a Plan Apochromat 100×/1.46 oil immersion lens.

As shown in Fig. 1, ABCA1 in an infected (gp120-positive) cell localized predominantly to intracellular regions that stain with calnexin-ER antibody, whereas in uninfected cells the majority of ABCA1 molecules resided at the plasma membrane, the site of active cholesterol exchange between ABCA1 and cholesterol acceptors in the blood.[4] These representative images were selected upon evaluation of several large tiled immunofluorescence images each containing over 100 cells. This relocalization of ABCA1 reflects its retention in the intracellular regions positive for calnexin, a marker for the ER. Such retention is consistent with Nef-mediated disruption of the calnexin–ABCA1 interaction, and may be responsible for subsequent degradation of ABCA1 and impairment of ABCA1-dependent cholesterol efflux.

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Author Disclosure Statement

No competing financial interests exist.

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