Ultrastructural visualization of cellular high density lipoprotein (HDL) internalization utilizing correlative microscopy

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High density lipoprotein (HDL) exerts atheroprotective effects transporting redundant cholesterol from peripheral tissues to the liver, which is referred to as reverse cholesterol transport (RCT). It is generally accepted that cholesteryl-esters are transferred between cells and HDL by selective uptake without HDL catabolism. Recently, we have identified HDL holo-particle uptake as an alternative pathway to regulate cellular cholesterol homeostasis [1]. The aim of the current study is the ultrastructural analysis of HDL holo-particle uptake, focusing on the intracellular compartments involved.

For correlative microscopy, diaminobenzidine (DAB)-photooxidation was utilized to convert fluorescent signals into electron dense reaction products visible in the transmission electron microscope (TEM) (see [2] for review). For HDL localization, HepG2 cells were incubated with fluorescent HDL-Alexa^{568} and analyzed by fluorescence microscopy (Fig. 1a). A parallel set of cells was fixed, DAB-photooxidation was performed [3] and samples were examined in the TEM.

After incubation with HDL-Alexa^{568} and subsequent photooxidation, TEM analyses revealed that HDL was localized in early endosomal compartments and multivesiculated bodies (MVBs; Fig. 1b), but not in late endosomes and lysosomes. Consistently, HDL did not colocalize with LIMP-II, as shown by confocal microscopy. HDL positive MVBs were found in the close vicinity of the *trans*-Golgi network (Fig. 1c); however, no contacts were observed. Confirmatively, no colocalization of HDL and TGN-46 was detected. To evaluate the internalization patterns revealed by DAB-photooxidation, cells were incubated with horseradish peroxidase (HRP)-tagged HDL and analyzed in the TEM, which resulted in comparable findings (Fig. 1d). Finally, selected HDL-positive endocytic compartments were 3-dimensionally reconstructed utilizing electron tomography.

1. Pagler et al., J Biol Chemistry **16** (2006), p11195-11204.
2. Meißlitzer-Ruppitsch et al., J Microscopy, in press.
3. Meißlitzer-Ruppitsch et al., Histochem Cell Biology **130** (2008), p407-419.
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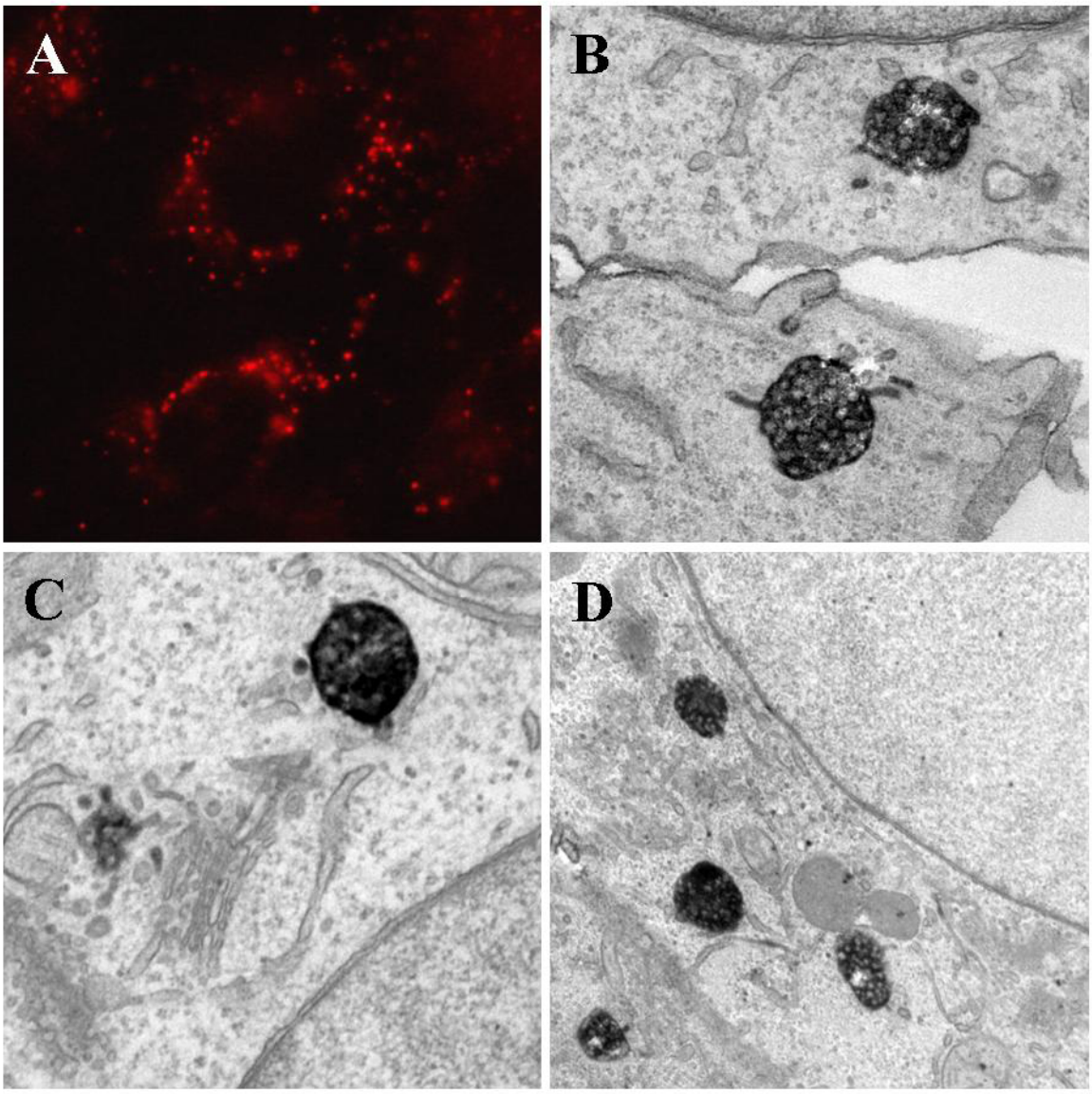

Figure 1. HepG2 cells were incubated with HDL- Alexa^{568} for 3 hours and imaged by fluorescence microscopy (A). A parallel set of cells was further processed for DABphotooxidation and TEM. HDL was localized preferentially in MVBs (B) and often in the close vicinity of the TGN (C). Localization of HDL with the well established HRP labelling (D) resulted in comparable findings. Magnifications were 100x (A), 12.000x (B,C) and 7.000x (D), respectively.

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