SR-BI mediated HDL endocytosis leads to HDL resecretion facilitating cholesterol efflux

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Introduction

The HDL receptor, scavenger receptor class B, type I (SR-BI), plays an important role in reverse cholesterol transport (RCT). SR-BI is responsible for the delivery of HDL derived cholesteryl-esters to liver and steroidogenic tissues in a process called selective cholesteryl ester uptake. The exact molecular mechanism by which HDL is loaded with cholesterol and delivers its cholesterol load to cells is however not completely understood. Especially the relevance of HDL holo-particle uptake for cholesterol exchange is still under research.
HDL holo uptake was first described by Schmitz et al. (1) in 1985 using macrophages. It was reported that HDL is taken up as an intact particle, located to non-lysosomal compartments and subsequently resecreted. This pathway was termed retroendocytosis. Recently SR-BI was described as a receptor mediating HDL endocytosis (2, 3). Silver et al. (2) demonstrated that in SR-BI transfected CHO cells and in primary hepatoma cells HDL holo-uptake occurs, HDL cholesterol ester depleted particles are released and that SR-BI is very likely to be the mediating receptor. However, the question of the physiological relevance of HDL endocytosis still remains as studies by Nieland et al. (4,5) suggest that selective uptake is not tightly linked or dependent on HDL particle uptake.

Aims

To analyze HDL holo-particle uptake, its relation to selective cholesteryl ester uptake and the role of SR-BI for this uptake process.

To investigate HDL resecretion and its role for cholesterol efflux.

Methods

Measurement of HDL holo-particle uptake and resecretion

Association – incubation of cholesterol depleted cells using 10µg/ml ^{125}I-HDL for 1h at 37°C → analysis
↓ extensive washing

Displacement – with an 100x excess of HDL for 2h at 0°C → analysis
(= HDL uptake)
↓ extensive washing

Chase – with an 20x excess of HDL for 30min at 37°C → analysis
(= HDL resecretion)

For analysis cells were lysed after the experiment and the amount of radioactivity was determined in the lysate.
As an ***alternative method fluorescence*** labeled HDL (Alexa647-HDL) was used and analyzed by fluorescence activated cell sorting analysis (FACS). The lipid part of HDL particles was followed, using DiI, during the uptake and resecretion of HDL.

For ***cholesterol efflux*** studies cells were pre-labeled with 2µCi 3H-cholesterol for 24h and subsequently the association, displacement and chase procedure was performed.

Morphological studies were performed by labeling HDL to either with peroxidase or gold and visualized using electron microscopy.

Single dye tracing was used to follow Cy5-HDL uptake and resecretion. It was further used to concomitantly follow Cy5-HDL and EGFP-SRBI endocytosis.

Cell lines used: SR-BI overexpressing cells (**ldlA7-SRBI**) as well as several cell lines relevant for cholesterol metabolism like HepG2, Y1BS1 and a monocyte cell line, THP-1, which was differentiated towards macrophages using 160nm PMA for 4 days.

References

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(3) Rhode, S. et al., (2004) Cell Biochem. Biophys. 41, 343–356
(4) Nieland, T. J. et al., (2002) Proc.Natl. Acad. Sci. U. S. A. 99, 15422–15427
(5) Nieland, T. J. et al., (2004) J. Lipid Res. 45, 1256–126

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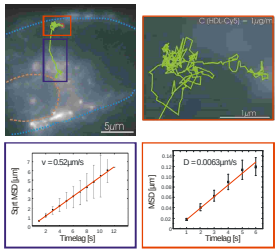
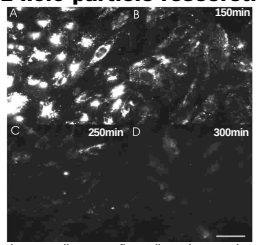
HDL holo-particle uptake



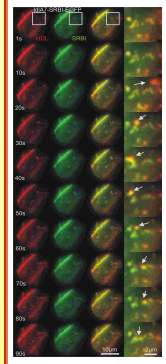
Uptake of HDL-Cy5 in a ldlA7-SRBI cell was followed as an intensity laps. The uptake of an individual HDL particle (upper right figure) shows trapping at the plasma membrane (red box) and directed transport (blue box) to the perinuclear region inside the cell. Analysis of such trajectories revealed an average diffusion constant of D = 0.004µm2/s and an average velocity of v = 0.43µm/s

HDL holo-particle resecretion



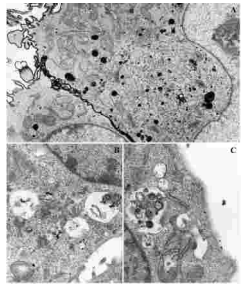
In this experiment cells were first allowed to endocytose HDL particles for 3h (typical staining pattern, A). After replacing the medium by a solution containing only unlabeled HDL, intracellular signals start to disappear. The amount of HDL particles inside the cells decreased dramatically (B-D), indicating recycling of HDL particles. As almost no cellular degradation of HDL particles occurs in ldlA7-SRBI cells endocytosed HDL particles must have been released.

Joint endocytosis of HDL and SR-BI



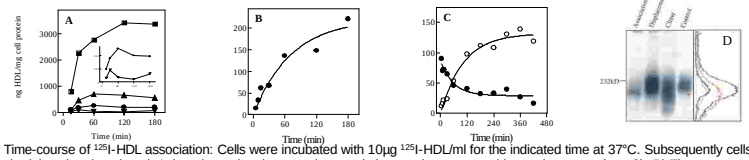
ldlA7-SR-BI-EGFP cells (green) were incubated with Cy5-HDL (1µg/ml; red) for 1h and then visualized using ultrasensitive microscopy for 90 sec with images taken every second. The green signal represents SR-BI-EGFP fusion protein;co-localization of SR-BI and HDL is shown in yellow. The right panel shows a higher magnification of the upper right part of the cell where co-localization leads to concomitant uptake of HDL with SR-BI, indicated by the arrow.

Intracellular HDL distribution



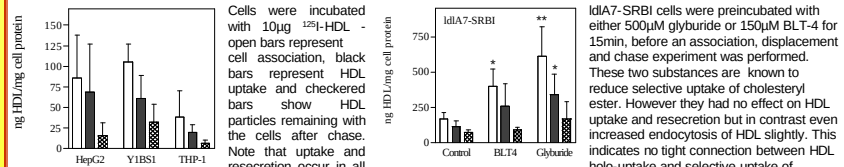
Electron microscopical localization of peroxidase- and gold-labeled HDL in ldlA7-SRBI cells: Peroxidase staining (A) and gold particles (B, C) were found inside endocytic vesicles and organelles. Magnifications of A, B and C are x13000, x25000 and x28500, respectively

HDL association, uptake and resecretion



A) Time-course of ^{125}I-HDL association: Cells were incubated with 10µg ^{125}I-HDL/ml for the indicated time at 37°C. Subsequently cells were washed, lyzed and analyzed. A time dependent increase in association can be seen reaching a plateau at about 2h. B) Time-course of ^{125}I-HDL uptake: cells were incubated with 10µg ^{125}I-HDL/ml for the indicated time. Then cells were washed, incubated with a 100 fold excess of unlabeled HDL at 0°C for 2 hours, lyzed and analyzed. A time dependent increase in uptake can be observed. C) Time-dependent exocytosis of ^{125}I-HDL: cells were incubated for one hour with 10µg ^{125}I-HDL, then with a 100 fold excess of HDL at 0°C for 2 hours. Finally cells were warmed to 37°C and chased with a 20 fold excess of HDL for different times. Media (○) and cells (●) were analyzed. Note the large increase in ^{125}I-HDL in the media and its concomitant decrease in the cell lysat. Values are expressed as specific association calculated by subtracting unspecific binding using a 40 fold excess of HDL from total cell association. D) Size fractionation of HDL, showing particle integrity in the media of association, displacement and chase.

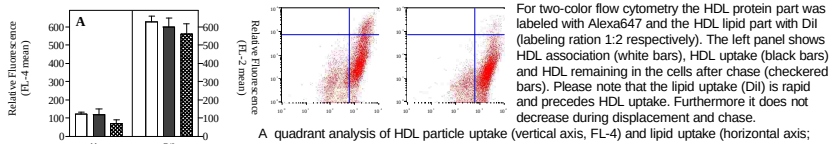
HDL retroendocytosis occurs in physiological relevant cell lines and is not tightly linked to selective cholesteryl ester uptake



Cells were incubated with 10µg ^{125}I-HDL - open bars represent cell association, black bars represent HDL uptake and checkered bars show HDL particles remaining with the cells after chase. Note that uptake and resecretion occur in all three cell lines.

ldlA7-SRBI cells were preincubated with either 500µM glyburide or 150µM BLT-4 for 15min, before an association, displacement and chase experiment was performed. These two substances are known to reduce selective uptake of cholesteryl ester. However they had no effect on HDL uptake and resecretion but in contrast even increased endocytosis of HDL slightly. This indicates no tight connection between HDL holo-uptake and selective uptake of cholesteryl ester.

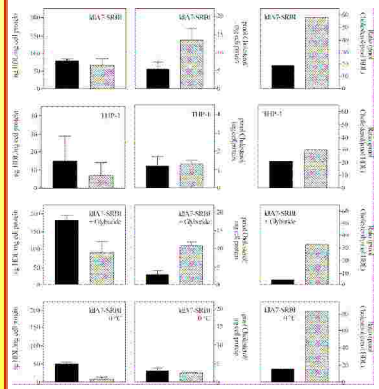
HDL particle uptake and resecretion differs from HDL-DiI lipid transfer



For two-color flow cytometry the HDL protein part was labeled with Alexa647 and the HDL lipid part with DiI (labeling ration 1:2 respectively). The left panel shows HDL association (white bars), HDL uptake (black bars) and HDL remaining in the cells after chase (checkered bars). Please note that the lipid uptake (DiI) is rapid and precedes HDL uptake. Furthermore it does not decrease during displacement and chase.

A quadrant analysis of HDL particle uptake (vertical axis, FL-4) and lipid uptake (horizontal axis; FL-2) after uptake (middle) and chase (right) is shown. Please note that the double labeled fraction decreased after the chase substantially, moving towards the lower left quadrant representing cells with incorporated DiI only.

HDL resecretion facilitates cholesterol efflux



Before the experiment, the cellular cholesterol pool was trace-labeled with 2 µCi of ^{3}H-cholesterol per well. On the experimental day, cells were incubated with 10 µg of ^{125}I-HDL/ml for 1 h with or without 500 M glyburide at 37 °C (upper panels) or at 0 °C (lowest panel) and then incubated at 0 °C for 2 h with a 100-fold excess of HDL (gray bars). Another set of cells was further treated with a 20-fold excess of HDL at 37 °C for 30 min (checkered bars). Media were collected, and aliquots were analyzed for their ^{3}H and ^{125}I content. The left panels show the HDL content in the media; middle panels represent the ^{3}H-cholesterol content in the media; the right panels represent the molar ratio of ^{3}H-cholesterol to ^{125}I-HDL protein (HDL 280 kDa) found in the media of ldlA7-SRBI cells and THP-1 cells. Please note the increase in the amount of cholesterol within HDL particles during the chase experiment. Lowering the temperature to 0°C during the initial incubation diminished the uptake of HDL particles and consequently diminished also the efflux of ^{3}H-cholesterol during the chase as no HDL particles were resecreted. This indicates that exocytosed HDL is loaded with cholesterol. Using glyburide a substance known to block selective uptake of cholesteryl ester we still could measure HDL uptake, resecretion and also cholesterol efflux, showing that this here described cholesterol efflux pathway is not connected with selective uptake. This novel cholesterol efflux facilitated by resecreted HDL particles could be important in genetic disorders where normal excretion of cholesterol does not function, as we could observe a similar HDL retroendocytosis and cholesterol efflux in fibroblast from Tangier patients.

Conclusion

- **HDL holo-particle uptake is mediated by SR-BI**
- **SR-BI is in part endocytosed together with HDL**
- **HDL holo-particle uptake occurs in physiologically relevant cell lines**
- **HDL holo-uptake and selective CE uptake are not tightly linked**
- **HDL resecretion can facilitate cholesterol efflux**

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