Modified low density lipoprotein delivers substrate for ceramide formation and stimulates the sphingomyelin-ceramide pathway in human macrophages

Ralf Kinscherf[1,b], Ralf Claus[1,a], Hans P. Deigner[a,*], Olaf Nauen[a], Christoph Gehrke[a], Albin Hermetter[e], Stefan Rußwurm[f], Volker Daniel[c], Volker Hack[d], Jürgen Metz[b]

[a] *Pharmazeutisch-Chemisches Institut, Universität Heidelberg, 69120 Heidelberg, Germany*
[b] *Institut für Anatomie und Zellbiologie III, Universität Heidelberg, 69120 Heidelberg, Germany*
[c] *Institut für Immunologie, Universität Heidelberg, 69120 Heidelberg, Germany*
[d] *Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany*
[e] *Institut für Biochemie und Lebensmittelchemie, Technische Universität, 8010 Graz, Austria*
[f] *Klinikum der Friedrich-Schiller-Universität Jena, 07740 Jena, Germany*

Received 27 December 1996

Abstract Exposure of human blood monocytes derived macrophages to modified (oxidized or acetylated) LDL induced a ~40% elevation (60 pmol/10^6 cells) of the endogenous level of the sphingolipid ceramide. A rise of both neutral and acidic SMase activity was found after treatment with oxidized LDL (250 and 80%), while addition of acLDL stimulated only the neutral enzyme (280%). Sphingo(phospho)lipids from LDL were transferred to the cell membrane and distributed into intracellular compartments as observed with acLDL containing BODIPY-FL-C_5-SM. Quantitation of ceramide after the addition of [^{3}H-*N*-acetyl]- or BODIPY-FL-C_5-SM-labeled modified LDL (27 μg/ml) to the cell culture medium indicated that approximately 210 pmol CA/10^6 cells was generated from exogenous (ox/acLDL) SM. These results demonstrate a stimulation of the sphingomyelin-ceramide pathway by modified LDL utilizing primarily exogenous (LDL-derived) substrate and suggest that the effects of modified LDL are at least partially due to an increased level of the messenger ceramide.

Key words: Macrophage; Lipoprotein; Oxidized LDL; Sphingomyelin; Ceramide

1. Introduction

Low density lipoprotein (LDL) is a (phospho)lipid-protein complex susceptible to oxidation which is generally considered to play a critical role in atherogenesis [1,2]. In plasma, the lipoprotein is an important carrier of phospholipids and neutral lipids. Exposure of monocytes/macrophages to LDL, in particular to chemically or oxidatively modified lipoprotein, causes a variety of biological effects and provokes a complex assembly of cellular responses including activation of distinct signal transduction pathways [3–6]. Among other phospholipids, LDL contains a considerable amount of sphingomyelin (SM) [7]. Ceramide (CA) generated by SM hydrolysis is increasingly recognized as an important mediator implicated in a variety of physiological processes such as cell growth, differentiation, and apoptosis [8,9]. The SM-CA pathway represents a route to transduce signals from cell surface receptors to the nucleus utilizing the diffusible lipid CA as a messenger [10,11]. Signal transduction in cells is induced by the hydrolysis of SM located in the plasma membrane, by a SM-specific phospholipase C, sphingomyelinase (SMase). Two forms of this enzyme are distinguishable by their acidic or basic pH optimum (review in [12]).

Although many mechanisms in the concert of cellular events induced by LDL and modified LDL have so far remained unclear, several effects could be assigned to lipid and phospholipid components of the particle [13]. We therefore reasoned that exogenous SM provided by LDL/modified LDL may affect the intracellular SM/CA pool in macrophages and may also alter concentrations of cellular CA and enter the CA signaling pathway. We show here that modified LDL delivers a substantial amount of SM substrate for CA generation by human blood monocytes derived macrophages (HBMC) and stimulates CA formation from endogenous SM.

2. Materials and methods

2.1. Materials

Human umbilical vein endothelial cells (EC, EndoPack-UV cell culture system), Clonetics (San Diego, CA); sphingomyelin phosphodiesterase (*E. coli*, ~220 units/mg), Sigma (Deisenhofen, Germany); 1,2-DAG-kinase (*Bacillus cereus*, 6.3 units/mg), Calbiochem (San Diego, CA); [γ-^{32}P]ATP (111 TBq/mM) Hartmann Analytic (Braunschweig, Germany); [^{3}H]acetic anhydride (18.5 GBq/mM, 3.7 GBq/ml) Amersham (Braunschweig, Germany); BODIPY-FL-C_5-SM/CA, Molecular Probes (Eugene, OR); other reagents, Sigma and Fluka (Neu-Ulm, Germany).

2.2. Cell culture

HBMC were prepared by Ficoll-Paque density gradient centrifugation from venous blood of healthy male donors according to published procedures [14,15] and cultured in RPMI 1640 medium supplemented with 10% heat inactivated FCS, glutamine (2 mM), and penicillin (100 units/ml), streptomycin (100 μg/ml) in a humidified

*Corresponding author. Fax: (49) (6221) 54 6430.

[1] Both authors contributed equally.

Abbreviations: acLDL, acetylated low density lipoprotein; BODIPY-FL-C_5-SM, *N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-sphingosylphosphocholine/sphingomyelin; BODIPY-FL-C_5-CA, *N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-sphingosine/ceramide; CA, ceramide; C_2-CA, *N*-acetyl-ceramide, *N*-acetyl-D-erythrosphingosine; DAG, diacylglycerol; EC, human umbilical endothelial cell(s); EC-LDL, LDL oxidized by endothelial cells; HBMC, human blood monocytes derived macrophages; oxLDL, oxidized LDL; PBS, phosphate-buffered sodium chloride solution; SM, *N*-acylsphingosin-1-phosphocholine, sphingomyelin; SMase, sphingomyelinase(s), sphingomyelin phosphodiesterase; TLC, thin-layer chromatography

PII S0014-5793(97)00157-9

atmosphere (5% CO_2 in air). The medium was changed daily. Non-adherent cells were removed by washing with supplemented medium and resident cells were used after 6 days. Prior to experimentation cells were plated in 6-well plastic cell culture plates (3×10^6 cells/well) and incubated for 3 h.

2.3. Preparation of LDL and modified LDL

LDL was isolated from pooled human plasma as described [16] and processed further as outlined previously [5]; all protein values refer to 500 kDa (apoB). Acetylated LDL (acLDL) was prepared according to the method of Basu et al. [17], oxidized LDL (oxLDL) by exposure to copper at reported conditions [5]. EC-LDL was obtained by incubation of confluent endothelial cells with LDL (100 μg/ml) for 24 h as described by Auge et al. [18].

2.4. Synthesis of [^{3}H-N-acetyl]sphingomyelin

[^{3}H-*N*-Acetyl]sphingomyelin was synthesized from phosphorylsphingosine (4 mg, 9 μmol) and [^{3}H]acetic anhydride (5.1 mg, 50 μmol, 18.5 GBq/mM (=3.7 GBq/ml toluene)) by reaction in toluene (18 h, RT) in the presence of *N,N'*-dicyclohexylcarbodiimide (41 mg, 200 μmol), (*N,N*)-diethylamine (10 μl) and diethylaminopyridine (2.7 mg, 20 μmol). The mixture was then stirred (40°C, 1 h) with 2 ml KOH (0.2 M) and tetrabutylammoniumhydroxide (cat.) to cleave acetyl esters, the organic phase separated, the aqueous phase extracted (chloroform, 3×3 ml) and the combined organic phases purified by silica gel chromatography (5 cm^3 silica gel grade 60, 70–230 mesh, elution of unpolar side-products by 15 ml chloroform then elution of product with chloroform/methanol/water 30:20:5, v/v (in the 15–20 ml fraction)). Further purification was achieved by TLC chromatography (0.5 mm plates) in the same eluent, scraping of the corresponding radioactive spot identified by nonradioactive standard and methanol extraction (3×4 ml). The product (1.5 mg, 2.96 μM, 9.2 GBq/mM, 33% yield referring to phosphorylsphingosine) was homogeneous in the above mentioned TLC system and showed a radioactive purity of ≥97%; identity was confirmed by FAB-mass spectroscopy.

2.5. Labeling of LDL with [^{3}H-N-acetyl]sphingomyelin and BODIPY-FL-C_5-sphingomyelin

Labeled SM ([^{3}H-*N*-acetyl]SM or BODIPY-FL-C_5-SM) was dissolved in chloroform (1 mg/ml); 10 μl of this solution was dried in a stream of nitrogen, sonicated (2 min, 4°C) in 100 μl Tris-HCl buffer (pH 7.5, 10 mM) and added to LDL or acLDL (1 ml, 1.28 mg). The mixture was treated (2 h, 37°C) with a plasma fraction as described [19] to catalyze the incorporation of labeled phospholipids. Labeled LDL was obtained after reisolation by density gradient centrifugation and dialysis at the above mentioned conditions. Result of a typical experiment: [^{3}H-*N*-acetyl]SM-LDL 95.7 kBq/mg, [^{3}H]SM-oxLDL 107.2 kBq/mg, [^{3}H]SM-acLDL 133.9 kBq/mg. In any preparation, the content of incorporated labeled SM in the lipoprotein was <2% of the natural content of SM (average value 181±32 molecules/particle (LDL concentration calculated on the basis of 500 kDa protein)); agarose gel electrophoresis indicated that lipoproteins labeled with [^{3}H-*N*-acetyl]SM or BODIPY-FL-C_5-SM migrated at the same position as the untreated particles.

2.6. Sphingomyelin/ceramide assays and sphingomyelinase activity

The total concentration of internalized/cell-associated ceramide was determined from cell extracts (treated with 30 μl 0.2 M KOH, 1 h, 37°C to hydrolyse DAG/esters) as [^{32}P]ceramidephosphate after incubation with [γ-^{32}P]ATP/DAG-kinase, subsequent extraction, TLC separation, scraping of phospholipids identified by standards and quantitation by counting of Cerenkov radiation (or by liquid scintillation counting when [^{3}H-*N*-acetyl]SM-lipoproteins were used) as described [20]; [^{3}H]C_2-CA, [^{3}H]SM content in cells (after incubation with [^{3}H-*N*-acetyl]SM-(ox/ac)LDL) is calculated as *N*-acetyl(phospho)lipid based on the respective specific activity of [^{3}H]SM in the lipoprotein, referring to an average SM content of 181±32 molecules/particle in the lipoproteins. The DAG-kinase method was also used for determination of SM in cells and CA/SM in lipoproteins (CA content was ≤6% in LDL oxidized by Cu^{2+} or EC, data are corrected with free (LDL-associated) CA present in the medium), SM content was assayed after incubation with SMase (approximately 40 mU/ml, 2 h, 37°C in buffer for neutral SMase as described [21] and data correction with CA values obtained without SMase treatment). BODIPY-FL-C_5-SM/CA concentration was determined after extraction and TLC separation at conditions outlined above, quantitation (a calibration curve was prepared) was obtained by fluorescence photometry (λ_{Ex} 503 nm, λ_{Em} 509 nm in methanol) after scraping of fluorescent spots and extraction with 3×5 ml of chloroform/methanol 2:1. Selective determination of the neutral/acidic SMase activity in cells was performed with [^{3}H]C_2-ceramide as a substrate at conditions described by Wiegmann et al. [21].

2.7. Fluorescence microscopy

Cells were treated with BODIPY-FL-C_5-SM-labeled lipoproteins (27 μg/ml, 1 h), washed three times with PBS (pH 7.4) and placed under a microscope (confocal laser-scanning microscope DM RB/E; Kr-line, λ_{Ex} 568 nm, λ_{Em} 602 nm with image documentation TCS4D; Leica, Bensheim, Germany).

3. Results

3.1. Uptake of phospholipids from [^{3}H-N-acetyl]sphingomyelin/BODIPY-FL-C_5-sphingomyelin-labeled LDL

In order to trace SM in lipoproteins and cells, LDL and acetylated LDL were labeled with [^{3}H-*N*-acetyl]SM or with BODIPY-FL-C_5-SM by means of plasma transfer proteins. After addition of [^{3}H]/BODIPY-FL-C_5-SM-labeled lipoprotein, the internalized/cell-bound label was quantitated. As shown in Table 1, modification of the LDL particle by oxidation or acetylation is followed by an enhanced uptake of ^{3}H-labeled (phospho)lipid from the particle. In comparison with [^{3}H]SM-LDL, 63% more tritiated material was internalized from [^{3}H]SM-acLDL and 46% from [^{3}H]SM-oxLDL after 1 h. About 4% of the total ^{3}H-radioactivity present in [^{3}H]SM-acLDL was found to be cell-associated already within 5 min, 6% after 20 min and 8% after 40 min (not shown) suggesting a rapid (phospho)lipid transfer in the initial phase. A preincubation with the scavenger receptor competitor poly-

Table 1
Uptake of ^{3}H-labeled (phospho)lipids from [^{3}H-*N*-acetyl]SM lipoproteins and effect on sphingomyelinase activity

[^{3}H]Lipoprotein	Uptake (%)	Sphingomyelinase activation (fold)	
		neutral	acidic
LDL	5.56±0.67	1.6	0.7
oxLDL	8.12±0.97	3.5	1.8
acLDL	9.07±0.93	3.8	0.8
acLDL+Poly I (10 μg/ml)	3.87±0.72	2.1	1.1

Human macrophages (3×10^6) were incubated with [^{3}H]SM-LDL (27 μg/ml, 1 h) or pretreated with polyinosinic acid (10 μg/ml, 30 min, washed (3×) with medium prior to the addition of the lipoprotein). The medium was removed, the cells were washed with precooled PBS and taken up into 50 μl homogenization buffer containing 0.1% Triton [21]. Radioactivity was measured in the supernatant of broken cells after centrifugation (4 min, 15000×*g*, 4°C). The uptake of cell-associated ^{3}H-labeled (phospho)lipid is calculated as percent [^{3}H-*N*-acetyl]SM, referring to the total radioactivity added with the lipoprotein (=100%); data are means of two experiments performed in triplicate. The change of neutral and acidic SMase activity (upon exposure to the respective LDL) is expressed relative to untreated controls (means of two experiments performed in duplicate, determined as described in Section 2).

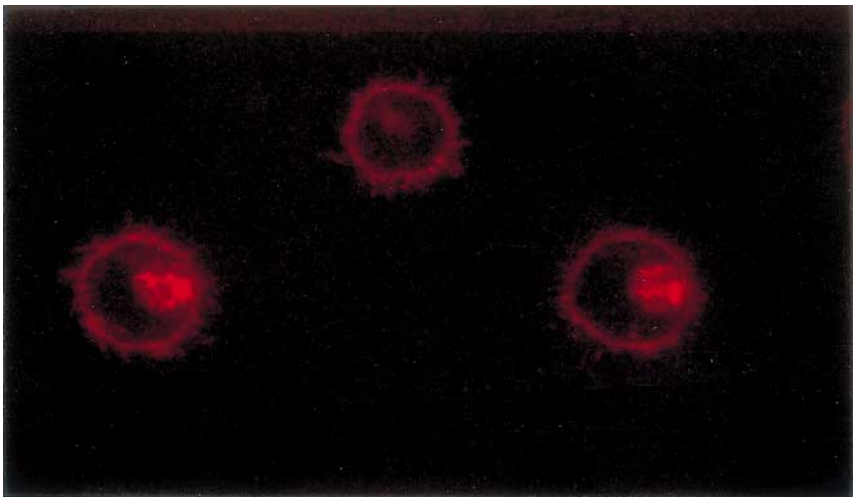

Fig. 1. Cellular distribution of BODIPY-FL-C_5-SM-labeled acLDL. Macrophages were treated with acLDL labeled with BODIPY-FL-C_5-SM (1 h, 27 μg/ml) and the distribution of fluorescent (phospho)lipids visualized by confocal laser scanning microscopy (1000-fold magnification, λ_{Ex} 568 nm, λ_{Em} 602 nm); fluorescent (phospho)lipids are localized on the cell surface/membrane, a granular staining is visible in the cytoplasm; typical result of three independent experiments.

inosinic acid (or fucoidan, 10 μg/ml, not shown) reduced the uptake of tritiated (phospho)lipid approximately to values obtained with [^{3}H]SM-acLDL.

Labeling with fluorescent BODIPY-FL-C_5-SM-LDL/acLDL was used to observe the cellular distribution of lipoprotein-bound (phospho)lipids in the cells. After 1 h incubation with 27 μg/ml modified lipoprotein, fluorescent compounds were distributed in the cell membrane as well as into intracellular compartments (Fig. 1). Distribution and relative intensity of the internalized/cell-bound fluorescence after addition of BODIPY-FL-C_5-SM-acLDL could not be distinguished from the partitioning of fluorescence obtained after incubation with a corresponding BODIPY-labeled LDL preparation.

3.2. Metabolism of LDL-sphingomyelin

HBMC were treated with [^{3}H-*N*-acetyl]SM-LDL, -acLDL, -oxLDL or the corresponding BODIPY-FL-C_5-SM-labeled lipoproteins (all 27 μg/ml) to examine the metabolism of SM derived from lipoproteins. The cells were washed, extracted and the labeled (phospho)lipids quantitated by liquid scintillation counting or fluorescence photometry after TLC separation. We observed that most of the (cell-associated) tritiated material was located in the [^{3}H]C_2-CA fraction (69 ± 17% after addition of acLDL, 65 ± 15% after addition of oxLDL, average values from three experiments), residual [^{3}H]SM added up to 9 ± 6% of the total radioactivity administered (not shown). According to the data depicted in Fig. 2, exogenous (initially lipoprotein-bound) SM was converted to ceramide to give 210 pmol [^{3}H]C_2-CA/10^6 cells (acLDL) or 93 pmol/10^6 cells (LDL). Comparable results were obtained when BODIPY-FL-C_5-SM-labeled LDL/acLDL was examined and BODIPY-FL-C_5-SM/CA analyzed by fluorometry (not shown). An increase in the [^{3}H]SM-acLDL concentration produced a concomitant rise in [^{3}H]C_2-CA which leveled off at a lipoprotein concentration of about 150 μg/ml (resulting in ~400 pmol CA/10^6 cells) indicating apparent saturation of uptake/cell association and/or enzymatic hydrolysis (Fig. 2, inset).

3.3. Effects of lipoproteins on cellular ceramide concentration and sphingomyelinase activity

Besides the portion of ceramide provided by the cleavage of LDL-SM, the level of CA derived from intracellular substrate (basal level 140 pmol/10^6 cells) was found to be increased after exposure to modified LDL (43% by acLDL, 35% by oxLDL; Fig. 3) and accompanied by a parallel decrease of SM from 420 to 360 pmol/10^6 cells (after acLDL treatment); the effect of LDL was marginal (~5%). The oxLDL-mediated effect was not restricted to lipoprotein prepared by Cu^{2+}-initiated in vitro oxidation as EC-LDL changed the percentage of CA similarly (Fig. 3). A significant reduction of the lipoprotein-induced SM hydrolysis (increase by 19%) was observed when the macrophages were incubated with polyinosinic acid prior to the addition of acLDL (Fig. 3). When the activity of the cleaving enzymes, the SMase, was assayed after the lipopro-

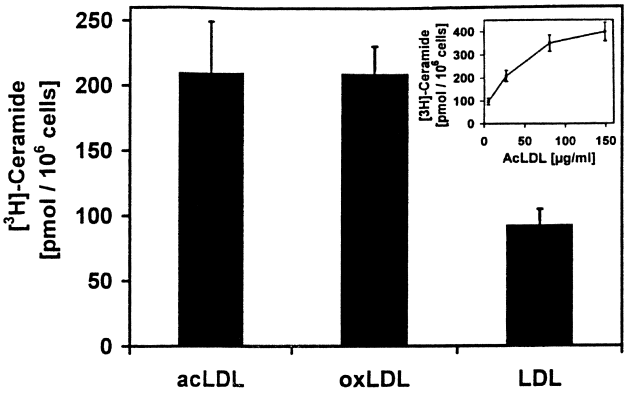

Fig. 2. Ceramide generation from lipoprotein derived sphingomyelin. Macrophages (3×10^6) were treated with [^{3}H-*N*-acetyl]SM-LDL, -acLDL or -oxLDL (60 min, 27 μg/ml), washed with PBS and broken by repeated freeze-thawing. Lipids were extracted and the amount of [^{3}H]C_2-CA determined after phosphorylation with DAG-kinase/[γ-^{32}P]ATP and TLC separation as described in Section 2. The dependence of the formation of [^{3}H]C_2-CA on the [^{3}H-*N*-acetyl]SM-LDL concentration is given in the inset. The data correspond to means ± S.E. of 3 separate experiments.

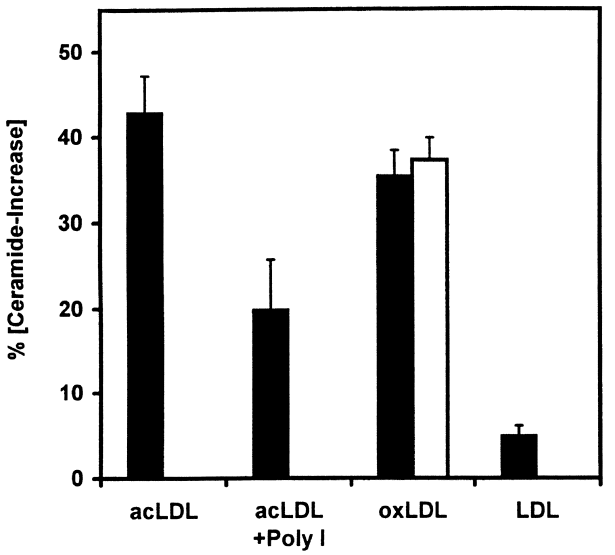

Fig. 3. Formation of ceramide from endogenous sphingomyelin in response to lipoprotein treatment. Ceramide concentration was measured after extraction of lipids, phosphorylation with DAG-kinase/[γ-^{32}P]ATP and TLC separation; the values represent the increase of ceramide (basal level 140 pmol/10^6 cells) found after incubation of cells with the lipoproteins (1 h, 27 μg/ml) and are corrected by the corresponding [^{3}H]C_2-CA values derived from exogenous (lipoprotein-associated) SM as determined with [^{3}H-*N*-acety]SM-labeled lipoproteins. The open bar gives the value obtained with EC-LDL (27 μg/ml). Results are given as means ± S.E. ($n = 3$).

tein treatment we found that the modified particles enhanced the activity of the neutral SMase by 280% (acLDL) and 250% (oxLDL) whereas the activity of the acidic SMase was only affected by the oxLDL treatment (+80%), a slight inhibition was noted after exposure to ac- or native LDL (Table 1). For either enzyme, the acLDL-induced change was markedly reduced by treatment with polyinosinic acid.

4. Discussion

Our results demonstrate that most of the sphingomyelin originating from LDL, acLDL or oxLDL undergoes conversion to ceramide by HBMC. The amount of ceramide produced depends on the lipoprotein concentration, correlates with the percentage of labeled material found cell-associated and coincides with a concomitant stimulation of SMase activity. Fluorescence microscopy provides evidence for the incorporation of LDL-SM into the acceptor membrane (an observation also made with platelets [22]) as well as into cytosolic compartments. In fact, Lipsky and Pagano [23,24] have shown that fluorescently tagged ceramides accumulate in the Golgi apparatus.

LDL modification increased the yield of ceramide produced from LDL-SM, an observation which could be explained by an enhancement of (phospho)lipid transfer from SR-bound particles and/or by an increased internalization/degradation. Conversely, a reduction of cell-associated CA was caused by pretreatment with the scavenger receptor competitors polyinosinic acid and fucoidan. However, although internalization of LDL-apolipoprotein is enhanced by modification [25], partitioning of LDL-phospholipids into the membrane of platelets apparently did not require endocytosis and lipid transfer was markedly faster than protein-cell association [22]. These data favor (phospho)lipid transfer rather than particle internalization. Besides scavenger receptor binding via modified apolipoprotein, stimulation of SMase(s) by modified lipoproteins may in turn increase aggregation and cell association [26,27].

Our current data indicate that the proportion of ceramide attributable to a cleavage of LDL-SM clearly exceeds that produced from endogenous substrate. When extracellularly bound ^{3}H-labeled acLDL was removed after 1 h by addition of an (15-fold) excess of unlabeled lipoprotein, cell-associated ^{3}H-radioactivity was reduced by 22% (R. Claus, personal communication) resulting in a ratio of 2.7:1 as for CA from exogenous/CA from endogenous SM (acLDL-derived CA diminished by 22%). Consistently, a significant amount of the diffusible metabolite CA may enter signalling pathways, even if cell-associated ceramide retained after washing may not completely be integrated into cellular compartments.

Auge and colleagues have shown that oxLDL induces SM hydrolysis in smooth muscle cells [18], a process which is accompanied by a decrease of the SM level and an increase of the CA level. Neutral SMase, an enzyme which resides at the membrane (although a Mg^{2+}-independent form has been identified in the cytosol as well [28]), is primarily activated by (modified) LDL and certainly contributes to LDL-SM cleavage. On the other hand, modified lipoprotein internalized via scavenger receptors is prone to degradation within lysosomes, and LDL-SM is thus a potential substrate of the acidic lysosomal SMase, compatible with the intracellular location of (initially lipoprotein-bound) BODIPY-FL-C_5-labeled (phospho)lipids. The acidic enzyme was stimulated by oxLDL treatment, not by acLDL which may be due to differences in the binding/uptake of the lipoproteins [29] and/or could reflect a distinct impact on redox-regulated processes. Accordingly, modified LDL is in principle capable of providing substrate for both neutral and acidic SMase, although, as concluded from activity changes, the role of the first mentioned enzyme may be more important. This issue however deserves further examination and Wiegmann et al. [21] have pointed out signal transduction by ceramide is dependent on the topology of formation and different pathways are controlled by distinct SMase activities. In this context, it is of note that Schütze and colleagues [30] and others [31] have suggested that acidic SMase can be activated by 1,2-DAG at the membrane; an induction of DAG increase by oxLDL in murine macrophages has been recently reported by us [5].

The results of our study show for the first time that in human macrophages LDL, in particular modified LDL, delivers SM substrate for CA generation, stimulates SMase activity and induces cleavage of cellular SM. A recent study states that retained lesional LDL accumulates ceramide [27], supporting the notion that our observations could have pathophysiological implications. Ceramide from lipoprotein sources may be involved in a number of regulatory events such as the induction of cyclooxygenase expression [32] and/or activation of MAP kinases [33,34], both effects observed with modified LDL [5,6]. It is now a matter of great interest to examine which of the various events induced by modified LDL in macrophages might be assigned to alterations in the SM-CA pathway.

Acknowledgements: This study was supported by the Deutsche Forschungsgemeinschaft (DFG Proj. No. DE 375/2-2) to H.P.D., and by

Grant 57103 (FWF) to A.H. Thanks are due to Elfriede Zenzmeier for excellent technical assistance, to Dr. A. Bryant-Friedrich for reading the manuscript and to Professor R. Neidlein for continuous encouragement.

References

[1] Ross, R. (1993) Nature 362, 801–809.
[2] Steinberg, D. (1995) in: Nutrition and Biotechnology in Heart Disease and Cancer (J.B. Longenecker, Ed.) pp. 39–48, Plenum Press, New York.
[3] Andalibi, A., Liao, F., Imes, S., Fogelman, A.M. and Lusis, A.J. (1993) Biochem. Soc. Trans. 3, 651–655.
[4] Lusis, A.J. and Navab, M. (1993) Biochem. Pharmacol. 46, 2119–2126.
[5] Claus, R., Fyrnys, B., Deigner, H.P. and Wolf, G. (1996) Biochemistry 35, 4911–4922.
[6] Deigner, H.P. and Claus, R. (1996) FEBS Lett. 385, 149–153.
[7] Esterbauer, H., Gebicki, J., Puhl, H. and Jürgens, G. (1992) Free Radical Biol. Med. 13, 341–390.
[8] Merrill. A.H. Jr., Hannun, Y.A. and Bell, R.M. (1993) Adv. Lipid Res. 25, 1–24.
[9] Kolesnick, R.N. and Fuks, Z. (1995) J. Exp. Med. 6, 1949–1952.
[10] Hannun, Y.A. (1994) J. Biol. Chem. 269, 3125–3128.
[11] Kolesnick, R.N. (1991) Prog. Lipid Res. 30, 1–38.
[12] Spence, M.W. (1993) Adv. Lipid Res. 26, 3–23.
[13] Zhang, H., Basra, J.K. and Steinbrecher, U.P. (1990) J. Lipid Res. 31, 1361–1369.
[14] Boyum, A. (1976) Scand. J. Immunol. 5, Suppl. 5, 9–16.
[15] Rußwurm, S., Krause, S., Finkelberg, L., Rühling, K., Schauer, F. and Lösche, W. (1994) Thromb. Res. 74, 505–514.
[16] Havel, R.H., Eder, H.A. and Bragdon, J.H. (1955) J. Clin. Invest. 34, 1345–1353.
[17] Basu, S.K., Brown, M.S., Ho, Y.K. and Goldstein, J.L. (1979) J. Biol. Chem. 254, 7141–7146.
[18] Auge, N., Andrieu, N., Negre-Salvyre, A., Thiers, J.C., Lavade, T. and Salvayre, R. (1996) J. Biol. Chem. 271, 19251–19255.
[19] Steinbrecher, U.P. (1987) J. Biol. Chem. 262, 3603–3608.
[20] Dressler, K.A. and Kolesnick, R.N. (1990) J. Biol. Chem. 265, 14917–14921.
[21] Wiegmann, K., Schütze, S., Machleidt, T., Witte, D. and Krönke, M. (1994) Cell 78, 1005–1015.
[22] Engelmann, B., Kögl, C., Kulschar, R. and Schaipp, B. (1996) Biochem. J. 315, 781–789.
[23] Lipsky, N.G. and Pagano, R.E. (1985) J. Cell. Biol. 100, 27–34.
[24] Lipsky, N.G. and Pagano, R.E. (1985) Science 228, 745–747.
[25] Via, D.P., Plant, A.L., Craig, I.F., Gotto, A.M. and Smith, L.C. (1985) Biochim. Biophys. Acta 833, 417–428.
[26] Tabas, I., Li, Y., Brocia, R.W., Wu, S.W., Swenson, T.L. and Williams, K.J. (1993) J. Biol. Chem. 268, 20419–20432.
[27] Schissel, S.L., Tweedie-Hardman, J., Rapp, J.H., Graham, G., Williams, K.J. and Tabas, I. (1996) J. Clin. Invest. 98, 1455–1464.
[28] Okazaki, T., Bielawska, A., Domae, N., Bell, R.M. and Hannun, Y.A. (1994) J. Biol. Chem. 269, 4070–4077.
[29] Lougheed, M. and Steinbrecher, U.P. (1996) J. Biol. Chem. 271, 11798–11805.
[30] Schütze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K. and Krönke, M. (1992) Cell 71, 765–776.
[31] Kolesnick, R.N. (1987) J. Biol. Chem. 262, 16759–16762.
[32] Ballou, L.R., Chao, C.P., Holness, M.A., Barker, S.C. and Raghow, R. (1992) J. Biol. Chem. 267, 20044–20050.
[33] Raines, M.A., Kolesnick, R.N. and Golde, D.W. (1993) J. Biol. Chem. 268, 14572–14575.
[34] Schütze, S., Machleidt, T. and Krönke, M. (1994) J. Leukocyte Biol. 56, 533–541.