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Interaction of Gangliosides with Plasma Low Density Lipoproteins

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The role of gangliosides in the reception of low density lipoproteins (LDL) was studied using as targets mouse ascites hepatoma 22a (MAH) cells which bind LDL through a specific high affinity receptor. Low density lipoprotein binding and uptake by MAH cells decreased after brief treatment of the cells with neuraminidase to partially remove surface sialic acid residues. The LDL uptake capability of the neuraminidase-treated MAH cells was fully restored after incorporation of exogeneous G_{M1}- and G_{D1a}-gangliosides into the cell surface. In contrast, free (extracellular) gangliosides inhibited LDL uptake by native MAH cells. This inhibitory effect was seen at ganglioside concentrations corresponding to the ganglioside content of serum and was most pronounced with gangliosides whose sialic acids were linked to a terminal galactose residue (G_{M3}, G_{D1a}, G_{T1b}) but was smaller or absent with gangliosides whose sialic acids were attached to an internal galactose (G_{M1}, G_{M2}). The binding of gangliosides to LDL was structure and concentration dependent, saturable and trypsin sensitive. The LDL-ganglioside interaction was further investigated by steady state fluorescence spectroscopy. Changes in the LDL fluorescence polarization were observed with as little as 0.01 μM concentrations of the gangliosides. The magnitude and nature of the effect depended on the type of ganglioside. We conclude that the LDL surface possesses sites recognizing specific carbohydrate sequences of glycoconjugates and that changes in the cell surface concentrations of sialic acids significantly modulate the LDL uptake. It is postulated that shedding of gangliosides into the blood stream may be a factor involved in regulation of cholesterol homeostasis.

Abbreviations: MAH, mouse ascites hepatoma 22a; LDL, low density lipoprotein; ASM, anthrylvinyl-labeled sphingomyelin [*N*-12-(9-anthryl-*trans*-dodecanoyl-sphingosine-1-phosphocholine]; RITC, rhodamine isothiocyanate. The designation of gangliosides follows the IUPAC-IUB recommendation [1]: G_{M3}, II^3NeuAc-LacCer, II^3-*N*-acetylneuraminosyllactosylceramide; G_{M2}, II^3-NeuAc-GgOse$_3$Cer, II^3-*N*-acetylneuraminosylgangliotriaosylceramide; G_{M1}, II^3-NeuAc-GgOse$_4$Cer, II^3-*N*-acetylneuraminosylgangliotetraosylceramide; G_{D1a}, II^3, IV^3(NeuAc)$_2$-GgOse$_4$Cer, II^3, IV^3-di(*N*-acetylneuraminosyl)gangliotetraosylceramide; G_{T1b}, II^3(NeuAc)$_2$,IV^3 NeuAc-GgOse$_4$Cer, II^3-di-*N*-acetylneuraminosyl, IV^3-*N*-acetylneuraminosylgangliotetraosylceramide.

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The low density lipoproteins are the major cholesterol carriers in plasma and high plasma levels of LDL are correlated with an increased occurrence of atherosclerosis [2]. The removal of LDL from the plasma is largely determined by LDL binding to specific LDL receptors [3] which are present on the surface of most mammalian cell types. Binding of LDL to the cell surface receptor is followed by internalization of the LDL particles and initiates a chain of events related to the metabolism of cholesterol (inhibition of cholesterol biosynthesis, stimulation of cholesterol esterification and inhibition of LDL receptor synthesis) [3]. Thus, regulation of LDL receptors is an important step in controlling cholesterol homeostasis. For this reason LDL receptorstudies have attracted much attention.

Since gangliosides are regular plasma components and are present on the surface of all mammalian cells, where they function as receptors or coreceptors for many types of ligands, the possible involvement of gangliosides in LDL reception is of considerable interest. Up to now this question has been studied only for human fibroblasts [4, 5]. Taking into account that the vast bulk of LDL receptors in animals are located in the liver [6, 7], the organ mainly responsible for cholesterol clearence, it appears important to elucidate the role of gangliosides in LDL uptake by hepatic cells. However, primary hepatocytes actively secrete lipoproteins [8, 9] which complicates receptor studies with these cells. To overcome such difficulties, attempts have been made to use as targets in LDL receptor studies human and murine hepatoma cells which secrete greatly reduced amounts of lipoproteins into the medium but still retain several differentiated functions of hepatocytes [10-12]. In order to investigate the role of gangliosides in the binding and uptake of LDL by hepatic cells we adopted as a model mouse ascites hepatoma cells, whose ganglioside composition previously has been studied in detail and which have been shown to bind LDL through a specific high affinity receptor [13, 14].

In the first part of this communication we describe the influence of modulating the ganglioside composition of MAH cells on LDL binding and uptake. (The term "uptake" refers to the net result of LDL binding, internalization and degradation at 37°C. "Binding" designates the cell association of LDL at 4°C, i.e. in the absence of internalization). In the second part we investigate the interaction of LDL with extracellular gangliosides.

Materials and Methods

Vibrio cholerae neuraminidase (500 units/ml) was purchased from Koch-Light Laboratories, Colnbrook, UK. One unit is the amount of enzyme required to liberate 1 μg of *N*-acetylneuraminic acid in 15 min from human serum glycoprotein at 37°C.

$Na^{125}I$ (carrier-free in 0.1 N NaOH) and sodium [^{14}C]acetate (50 Ci/mol) were from Amersham International, Arlington Heights, IL, USA. Heparin was purchased from Sigma Chemical Company, St. Louis, MO, USA. ASM was obtained from bovine brain sphingomyelin and 9-anthryl-*trans*-dodecenoic acid according to [15].

Gangliosides G_{M1}, G_{D1a} and G_{T1b} were isolated from human brain [16]; G_{M3}-ganglioside from human liver [17] and G_{M2}-ganglioside from mouse liver [13]. ^3H-Gangliosides were obtained by catalytic tritium exchange over 5% $Pd/BaSO_4$ [8].
Human LDLs (density 1.015-1.063 g/ml) were obtained from fresh plasma of normal volunteers by differential ultracentrifugation as described in [19] and their purity was checked by agarose-gel electrophoresis [20]. ^{125}I-LDLs was prepared by the iodine

monochloride method [21]. RITC-LDLs were prepared by rhodamine-isothiocyanate treatment of LDL [22].

Cells

Hepatoma 22a ascites cells were grown in the peritoneal cavity of male C3HA mice for seven days; the ascitic fluid drawn off from the peritoneal cavity contained 51 mg/ml of cellular protein, corresponding to 3×10^8 cells/ml. The ascitic fluid was centrifuged at $1000 \times g$ for 5 min. The cell pellet was washed three times with 199 medium (IPVE AMS USSR) and suspended in the medium to obtain a final concentration of 50 mg protein/ml.

Incubation of MAH Cells with Neuraminidase

Washed intact MAH cells were suspended in 5 ml of Hanks solution (IPVE AMS USSR) containing 1% bovine serum albumin to give a final concentration of 1×10^7 cells/ml. *V. cholerae* neuraminidase (125 units) was added and the suspension was then incubated for 1 h at 37°C. The incubation was terminated by three washings with the same medium. The content of total [23] and lipid-bound [24] neuraminic acid was determined as described.

Incubation of MAH Cells with Gangliosides

The neuraminidase-treated hepatoma cells were resuspended to a concentration of 1×10^6 cells/ml in Hanks medium containing unlabeled (50 nmol/ml) or tritium-labeled gangliosides (50 nmol/ml), 0.75 Ci/mmol) and were incubated with stirring at 37°C for 30-40 min. The cells were separated by centrifugation, washed three times and were then resuspended in Hanks medium. For determination of cell associated radioactivity, cell pellets were dissolved in 2 M NaOH, and the radioactivity was counted.

LDL Uptake Experiments

The uptake (binding, internalization and degradation) of LDL by MAH cells at 37°C was measured using methods previously described [14]. In brief, the cell suspension containing 1×10^6 cells/ml in 199 medium (0.6 ml) was incubated with various concentrations of ^{125}I-LDL (50-100 cpm/ng) or RITC-LDL for 1 h and then washed extensively. The cell radioactivity was measured in a gamma-counter (LKB, Bromma, Sweden, model 1282), and the fluorescence was determined using the FACS II flow cytofluorimeter (Becton Dickinson, Mountainview, CA, USA; Argon laser, fluorescence excitation 514 nm, emission 580 nm). In order to estimate the extent of specific and non-specific uptake of LDL, cell suspensions were incubated with ^{125}I-LDL or RITC-LDL and a ten-fold excess of unlabeled LDL. The non-specific uptake was substracted from the values for total uptake of LDL to obtain the specific uptake of ^{125}I-LDL or RITC-LDL. Unless otherwise noted, data of LDL binding or uptake experiments have been corrected for non-specific binding or uptake. To study the influence of extracellular gangliosides on LDL uptake 300 μg ^{125}I-LDL were incubated with 10 nmol of gangliosides at 37°C for 1 h in 199 medium. The uptake of ganglioside-treated LDL by MAH cells was determined as described above.

Synthesis of Cholesterol from [^{14}C]Acetate by MAH Cells

MAH cells (1×10^6 cells/ml) were incubated for 3-4 h at 37°C in medium containing [^{14}C] acetate (2.5 mM) and appropriate concentrations of LDL as described [25]. The cells were treated with ethanol and then 2 M aqueous NaOH, unsaponified lipids were extracted with hexane and separated by TLC on silica gel plates (Silufol, Merck, Darmstadt, W. Germany). The plates were developed with hexane/diethyl ether/acetic acid, 85/15/1 by vol. Cholesterol was visualized by spraying with ethanolic phosphomolybdic acid and subsequent heating. The cholesterol spots were scraped from the plate and the radioactivity was counted.

Binding of Gangliosides to LDL-Affinity Gel

LDL immobilized on CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) was prepared as described in [26]. The protein concentration of the affinity gel was 2 mg/ml. Various amounts of tritium-labeled gangliosides were added to the affinity gel (50 µl) in 1 ml of 1% $CaCl_2$ and the mixture was incubated for 30 min at 20°C. The gel was washed three times with 1% $CaCl_2$ and the radioactivity of the gel was counted. In order to estimate the extent of specific and non-specific binding the LDL-affinity gel was incubated with ^{3}H-gangliosides in the presence of a 100-fold excess of the corresponding unlabeled gangliosides. The influence of heparin on the ganglioside binding capacity of the LDL affinity gel was studied by performing the above binding experiments in the presence of excess heparin (2 mol heparin/mol ganglioside).

To determine the trypsin sensitivity of the ganglioside binding the LDL-affinity gel (100 µl), preincubated with ^{3}H-gangliosides (20 nmol/ml) and washed as indicated above, was treated with a 0.25% trypsin (Calbiochem, San Diego, CA, USA) solution in 0.02% EDTA (1 ml) for 30 min at 20°C. The solution was separated from the gel and after three washings with 1% $CaCl_2$ the residual radioactivity of the gel was counted in a beta-counter (model 1215, LKB). Controls were carried out with Sepharose 4B (Pharmacia) not containing LDL. In order to estimate the amount of ganglioside removable from the affinity gel, the gel preincubated with ^{3}H-gangliosides (20 nmol/ml, 200 000 dpm) was incubated with 3×5 ml of 0.5 M NaCl in 1% $CaCl_2$ for 30 min at 20°C. After decantation, the residual radioactivity of the gel was counted as indicated above.

Fluorescence Polarization Studies

Steady state fluorescence polarization was measured as described [27] using a Hitachi 650-60 spectrofluorimeter with a thermostated 5×5 quartz cuvette. The slit width was 2 nm for excitation and 10 nm for emission. Fluorescence polarization was calculated by the processor of the spectrofluorimeter. To a suspension of LDL in 0.9% NaCl (1 mg protein/ml) an ethanolic solution of ASM was added. The probe to phospholipid ratio was 0.01 and the final concentration of ethanol in the sample was less than 0.5%. The mixture was incubated for 3 h at 36.5°C, ganglioside (10 pmol/ml) was added and the incubation was continued for 1 h. The fluorescence polarization before and after addition of the ganglioside was recorded.

Unless otherwise noted all results shown in the present work are means of 3-5 independent experiments with coefficients of variation between 6.8 and 10.1%.

Table 1. Effect of neuraminidase treatment and subsequent ganglioside loading of MAH cells on the binding and uptake of RITC-LDL. 50 mg LDL was added to 10^6 cells. For other conditions see the text.
Results are expressed as a % of the control (native cells).

MAH-cells	Specific binding (4°C)	Specific uptake (37°C)
Native cells (control)	100	100
Neuraminidase-treated cells	20	60
Neuraminidase-treated cells subsequently loaded with:		
G_{M1}	119	120
G_{D1a}	181	172
Total brain gangliosides	190	159

Results

Influence of Neuraminidase Treatment and Ganglioside Loading on the Binding and Uptake of LDL by MAH Cells

Previous results [28] demonstrated the usefulness of flow-cytometry with fluorescence labeled LDL in receptor studies. However, that method does not permit the determination of quantitative characteristics of LDL-receptor interactions. For this reason both fluorescent (RITC) and ^{125}I-labeled LDL were used in the present investigation.

The level of sialoglycoconjugates in intact MAH cells corresponds to 2×10^{-6} nmol sialic acid/cell [14]. About half this amount (1.1×10^{-6} nmol/cell) was released on treatment of the cells with neuraminidase from *Vibrio cholerae*. Neuraminidase treatment inhibited the LDL binding and uptake capability of MAH cells to a different extent: binding was lowered by up to 80%, whereas uptake decreased only by 40% in comparison with native MAH cells (Table 1). After neuraminidase treatment, LDL binding to MAH cells remained predominantly specific; the association constant did not change significantly from 0.11×10^9 M^{-1} (Fig. 1). Neuraminidase-treated MAH cells were able to bind exogenous gangliosides; the binding of ganglioside G_{D1a} was concentration dependent and saturable (Fig. 2). After loading of neuraminidase-treated MAH cells with gangliosides G_{M1} or G_{D1a} the sialic acid content of the cells was 1.4 or 1.8 times higher than that of native cells, respectively. Loading with gangliosides markedly improved the specific LDL binding capability of neuraminidase-treated MAH cells to a level that was even higher than that of native MAH cells (Table 1 and Fig. 1). It is noteworthy, that neither neuraminidase treatment, nor subsequent ganglioside loading had any influence on the non-specific uptake of LDL by MAH cells (Fig. 3).

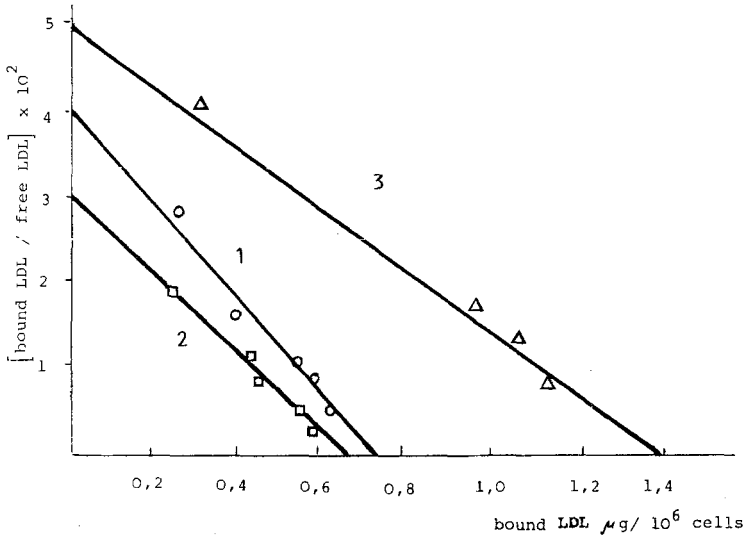

Figure 1. Scatchard plots for ^{125}I-LDL-specific binding by MAH cells (1); neuraminidase-treated cells (2); and neuraminidase-treated, ganglioside G_{D1a}-loaded cells (3).

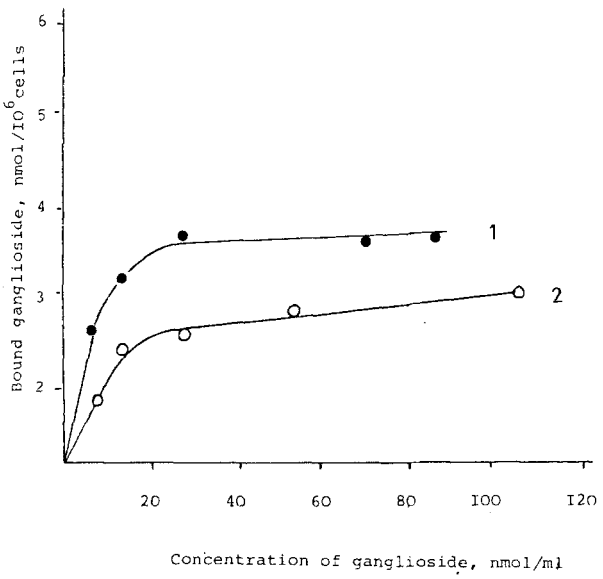

Figure 2. Saturation curve of ^{3}H-G_{D1a}-specific binding by native (1); and neuraminidase treated (2), MAH cells.

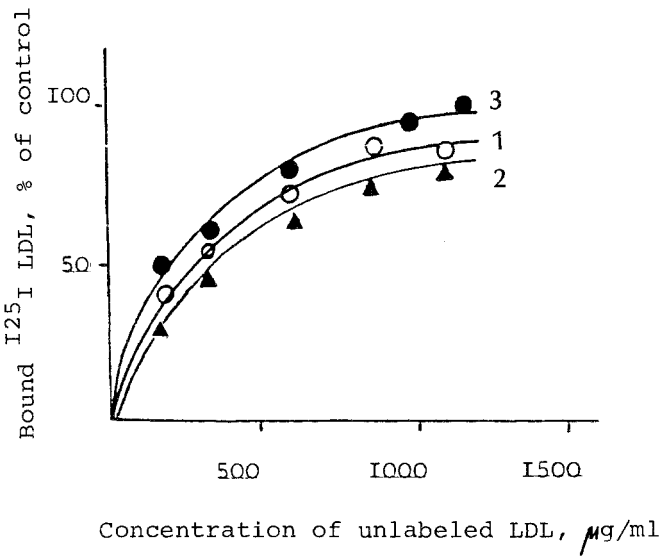

Figure 3. Non-specific LDL uptake by MAH cells. 10^6 cells/ml were incubated with 50-150 μg/ml of ^{125}I-LDL for 1 h at 37°C in the presence of a ten-fold excess of unlabeled LDL: native cells (1); neuraminidase-treated cells (2); neuraminidase-treated, G_{D1a}-loaded cells (3). 100% (control) corresponds to LDL uptake by cells at 1200 μg/ml ^{125}I-LDL in the medium.

In order to investigate the effect of LDL uptake by MAH cells on cholesterol biosynthesis we measured [^{14}C]acetate incorporation into cholesterol in the absence of LDL and in the presence of various LDL concentrations. As indicated in Fig. 4, LDL markedly inhibited the incorporation of [^{14}C]acetate into cellular cholesterol. The effect was concentration dependent and saturable. The concentration of native LDL causing maximal inhibition was lower than the saturation value of ^{125}I-LDL uptake (10-20 and 50-60 μg LDL protein per ml, respectively). This difference may be due to modification of the LDL structure during iodination.

Neuraminidase treatment of MAH cells abolished the inhibitory effect of LDL on cholesterol biosynthesis (Fig. 4). The LDL inhibitory effect could be largely restored to the neuraminidase-treated cells with ganglioside G_{D1a}.

Interaction of LDL with Gangliosides

Preincubation of LDL with gangliosides (20 nmol sialic acid/ml) markedly decreased the uptake of LDL by MAH cells (Table 2). The inhibitory effect was most pronounced with gangliosides containing a sialic acid at the non-reducing terminus (G_{M3}, G_{D1a}, G_{T1b}), whereas gangliosides containing sialic acid attached to an inner galactose residue (G_{M2}, G_{M1}) only weakly retarded LDL uptake. An analogous inhibitory effect was seen when LDLs were preincubated with total brain gangliosides.

In order to gain insight into the nature of the ganglioside-LDL interaction we determined the binding of various gangliosides to LDL immobilized on Sepharose 4B and studied the effect of gangliosides on fluorescent-labeled LDL.

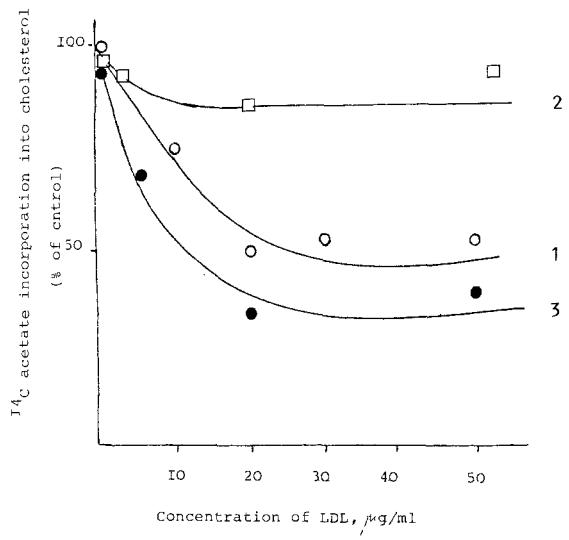

Figure 4. Effect of LDL and gangliosides on cholesterol biosynthesis in MAH cells. 10^6 cells/ml were incubated with [^{14}C] acetate (0.1 mCi/ml) and LDL for 3 h at 37°C: native cells (1); neuraminidase-treated cells (2); neuraminidase treated, G_{D1a}-loaded cells (3).

Table 2. Inhibitory effect of extracellular gangliosides on the specific LDL uptake by MAH cells.

Gangliosides added to the incubation medium	Specific LDL uptake (% of control)[a]
G_{M3}	70
G_{M2}	96
G_{M1}	97
G_{D1a}	70
G_{T1b}	74

[a] Control less LDL uptake in the absence of ganglioside. For experimental conditions see the Materials and Methods section.

Table 3. Ganglioside composition of the MAH cells [13].

Ganglioside	% of total
G_{M3}	8
G_{M2}	73
G_{M1}	11
G_{D1a}	5
G_{T1b}	3

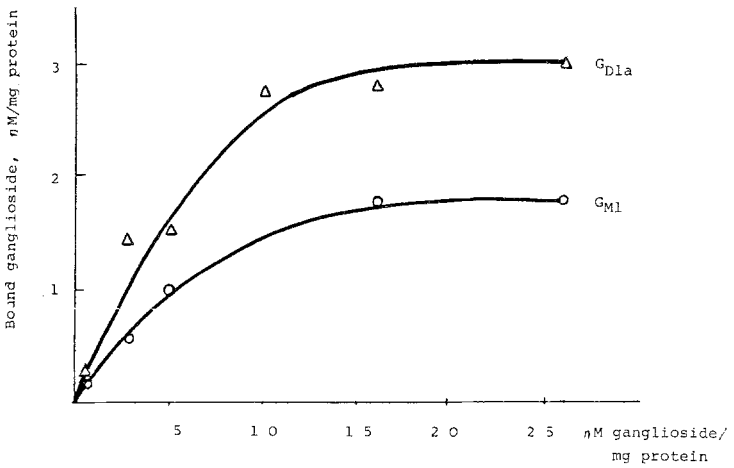

Figure 5. Concentration dependence of the binding of ^{3}H-G_{M1} and ^{3}H-G_{D1a} to LDL-affinity gel.

The binding of gangliosides G_{D1a} and G_{M1} to Sepharose-immobilized LDL was structure and concentration dependent (Fig. 5). Mild trypsinization of the ganglioside-loaded LDL affinity gel removed up to two thirds of the gangliosides bound (Table 4). Somewhat higher amounts of gangliosides were removed from the gel upon extensive washing with 0.5 N NaCl solution. Preliminary trypsinization of the affinity gel resulted in loss of more than 60% of the ganglioside binding activity. In the presence of excess heparin the binding of gangliosides G_{D1a} and G_{M1} to LDL was reduced by 50-80% (Table 4). The ganglioside binding of Sepharose 4B not containing LDL was about a third of that of the LDL affinity gel.

Small amounts of gangliosides G_{D1a} and G_{M1} had a pronounced effect on the fluorescence anisotropy of LDL labeled with the fluorescent sphingomyelin analogue, ASM. The ganglioside induced changes of the fluorescence polarization were concentration dependent, saturable (Fig. 6) and depended specifically on the structure of the ganglioside: G_{D1a} and G_{M1} caused oppositely directed changes whereas G_{M2} had no measurable effect (Fig. 7). With G_{D1a} the effect was saturated at 10^{-8} - 10^{-7} M, i.e. at concentrations corresponding to one or few ganglioside molecules per molecule of LDL. No fluorescence polarization changes were observed when the same small amounts of G_{M1} or G_{D1a} were added to ASM-labeled liposomes made from a lipid mixture whose composition simulated that of the LDL surface (egg phosphatidylcholine, brain sphingomyelin, cholesterol in a molar ratio of 2:1:1) (data not shown).

Table 4. Interaction of ^{3}H-gangliosides with LDL-Sepharose.

Ganglioside	Radioactivity added (cpm)	LDL-Sepharose-associated radioactivity (cpm)			
		total	after trypsinization	after washing with 0.5N NaCl	in presence of heparin
G_{M1}	200 000	35 000	23 000 (66)[a]	11 000 (31)	18 000 (51)
G_{D1a}	180 000	75 000	33 000 (44)	21 000 (28)	16 000 (21)

[a] The figures in brackets show % of total bound radioactivity.

Discussion

Our previous work demonstrated that MAH cells possess high affinity binding sites that recognize human LDL [14]. The LDL binding and uptake followed similar kinetics suggesting that binding leads to intracellular processing of the lipoproteins. This conclusion was indirectly confirmed by the fact that human LDL inhibited cholesterol synthesis in MAH cells in a manner similar to the inhibiting effect of these lipoproteins on cholesterol synthesis in hepatic cells of animals and man (see [29] for a review). In the liver, cholesterol synthesis is known to be under feedback control whereas such control is lost or impaired in various cancer cells, including hepatomas (reviewed in [30]). In contrast, our studies demonstrated a profound cholesterol feedback response to LDL uptake in MAH cells [14]. Recently, feedback regulation of cholesterol synthesis in response to LDL treatment was demonstrated also for human hepatoma cells [31]. Taking into account the similarity of LDL uptake in MAH and human cells, the ease of handling the former cells and their relation to the liver, we attempted to use MAH cells as a model in order to elucidate the role of gangliosides in binding and uptake of human LDL. Previous studies on the influence of sialoglycoconjugates on LDL-cell interaction have led to conflicting results. According to Filipovic *et al.* [4] the receptor-mediated uptake of LDL increases after desialylation of the lipoproteins. However, Attie *et al.* [32] were not able to detect any correlation between LDL-cell binding and the presence of sialic acids on the LDL surface. According to some authors [26], increasing the number of sialic acid residues on the surface of recipient cells stimulates receptor-mediated LDL uptake, whereas others found that the uptake increased after partial desialylation of the cell surface [33]. Some of these data deviate also from those of the present work. The differences may be due to the use of different recipient cells as well as different experimental conditions. Our results demonstrate that in MAH cells, LDL binding correlates positively with the number of sialic acid residues on the cell after partial desialylation of the cell surface. Interestingly, neuraminidase treatment of the cells inhibited LDL binding to a much greater extent then LDL uptake (Table 1). Hence the neuraminidase sensitive glycoconjugates of the cell surface appear to be of relatively more importance in LDL binding and of less importance in LDL internalization than are

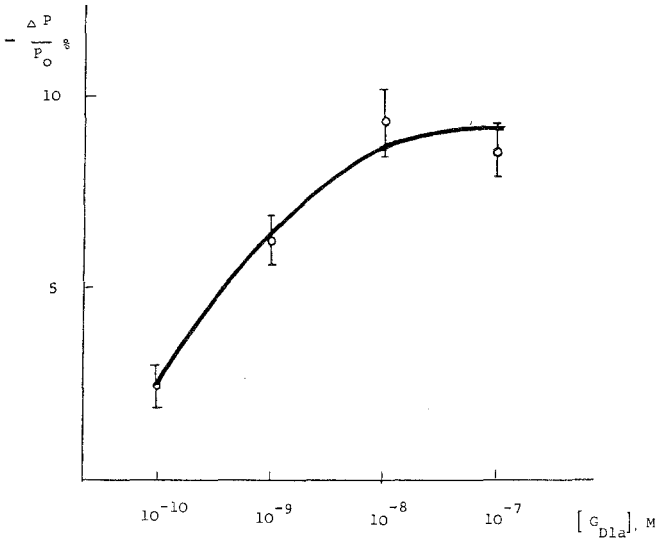

Figure 6. Concentration dependence of G_{D1a}-induced changes of the fluorescence polarization of ASM-labeled LDL. $\Delta P = P - P_0$ where P_0 and P are the fluorescence polarization values before and after addition of the ganglioside. For experimental conditions see the Materials and Methods section.

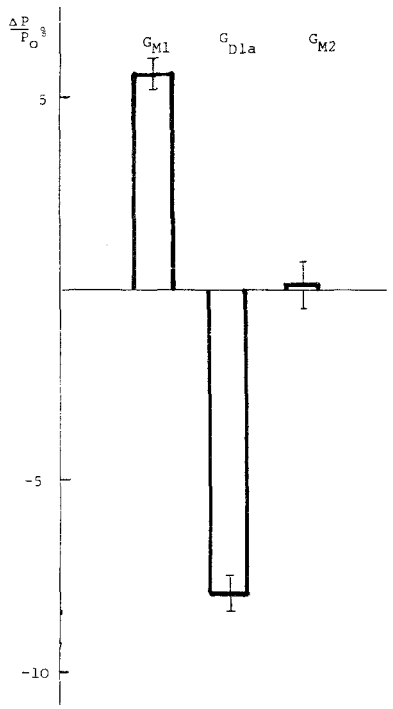

Figure 7. Ganglioside-induced changes of the fluorescence polarization of ASM-loaded LDL (ganglioside concentration 10^{-9} M). Designations as in Fig. 6. For experimental conditions see the Materials and Methods section.

neuraminidase-resistant cell surface structures. The latter include the gangliosides G_{M2} (the major ganglioside component of MAH cells, see Table 3) and G_{M1}. As has been shown earlier [34], G_{M1} is the end product of neuraminidase digestion of the three MAH gangliosides G_{D1a}, G_{D1b} and G_{T1b}. Thus, a major part of the lipid-bound sialic acid residues of MAH cells should survive the desialylation procedure, whereas the glycoproteins may be expected to be desialylated to a much higher extent. Therefore, the fact that the major part of the LDL uptake capacity of MAH cells is preserved after neuraminidase treatment suggests that cell gangliosides may be involved in LDL internalization. Indeed, incorporation of exogenous gangliosides G_{M1} and G_{D1a} into neuraminidase-treated MAH cells restored the LDL uptake of the cells to a level even exceeding that of the native cells. At the same time incubation of MAH cells with LDL in the presence of free ganglioside G_{D1a} (but not G_{M1}) markedly inhibited LDL uptake. Such behaviour may be explained by assuming the existence on the LDL surface of binding sites recognizing the carbohydrate moieties of specific gangliosides. Upon incubation in the presence of excess free gangliosides these sites are blocked. Conversely, preincubation of neuraminidase-treated cells with gangliosides results in incorporation of the lipophilic ceramide moieties into the lipid bilayer, thus increasing the number of LDL binding sites on the cell surface. The different mode of interaction of gangliosides with LDL and with MAH cells may be explained by differences in their surface structure. The phospholipids of the LDL outer monolayer are known to be packed much tighter that those of eucaryotic plasma membranes (reviewed in [35]). At the same time LDLs are characterized by a relatively high positive charge which increases under hypercholesteremic conditions [36-38]. These factors should facilitate the hydrophilic interaction of the negatively charged gangliosides with LDL and hinder incorporation of the hydrophobic moieties of the ganglioside molecules into the LDL outer monolayer. On the contrary, upon incubation of extracellular gangliosides with partly desialylated MAH cells, a significant part of the gangliosides may be expected to become associated with the plasma membrane due to hydrophobic incorporation of the ceramide moieties into the lipid bilayer. The supposition of a specific hydrophilic interaction of LDL with gangliosides was corroborated by direct binding experiments and fluorescence studies (Fig. 5 and 6). The fact that ganglioside G_{D1a}-binding to LDL is predominantly trypsin sensitive and that addition of gangliosides to ASM-labeled protein-free liposomes does not induce changes of their fluorescent polarization indicates that LDL is largely responsible for recognition and binding of the gangliosides.

In radioligand binding assays of LDL, the extent of specific binding may be evaluated by adding excess heparin to the incubation medium [34]. The amount of LDL which binds to cells under such conditions corresponds to non-specific interaction. When excess heparin was added to the LDL-affinity gel simultaneously with G_{D1a}, binding of the ganglioside was reduced by 65-70%. At the same time, binding of G_{D1a} to Sepharose 4B not containing LDL comprised about 30% of the binding of the same ganglioside to the LDL-affinity gel. We conclude that the binding of G_{D1a} to immobilized LDL is predominantly specific and hydrophilic. The fluorescence data of Fig. 6 indicate that even sub-physiological amounts of gangliosides may alter the LDL surface structure in a concentration dependent manner. An apparent discrepancy of our results could be perceived in the fact that free G_{M1} did not influence binding of LDL to intact MAH cells, whereas cell-bound G_{M1} was able to restore the uptake capability of neuraminidase treated cells (Tables 1 and 2). In contrast, G_{D1a} was effective in both cases. The reason for this difference is not known. It should be noted in this connection that the two

gangliosides induce oppositely directed changes in the fluorescent polarization of ASM-labeled LDL (Fig. 7) and thus appear to interact differently with the apoprotein.

When discussing the possible biological relevance of these data one should take into account that in our experiments ganglioside-mediated specific LDL uptake resulted in inhibition of cellular cholesterol synthesis. Hence, the amount and composition of gangliosides in LDL recipient cells may be a factor influencing cholesterol homeostasis.

Although our data do not prove that the receptor mediated pathway of LDL-cell interaction depends on gangliosides as an obligatory condition, they nevertheless indicate that gangliosides may be involved in some steps of this interaction. Possibly the first step of the interaction process, i.e. LDL binding to the cell surface, is put into effect by glycoproteins, whereas gangliosides are involved in subsequent internalization steps. After partial desialylation of the cell surface, the gangliosides seem to be able to ensure both binding and internalization of the LDL particles.

Of great potential interest is the ability of extracellular gangliosides to block the specific interaction of LDL with the recipient cells. Serum is known to contain appreciable amounts of gangliosides (about 20 nmol/ml) [39] due to shedding of membrane fragments into the bloodstream [24, 40, 41]. Since the shedding efficiency depends on the state of the plasma membrane, particularly the cholesterol content, it seems probable that inhibition of binding by gangliosides shed from the cell surface may be of regulatory significance. This supposition requires, of course, further examination.

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