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GANGLIOSIDE GM3 STIMULATES THE UPTAKE AND PROCESSING OF LOW DENSITY LIPOPROTEINS BY MACROPHAGES

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Preincubation of low densiy lipoproteins (LDL) with low concentrations of the ganglioside GM3 (1-2x 10^{-5} M/ 2.5x10^{-6} M LDL-protein) results in an increase of LDL-uptake, enhances cholesterol accumulation and cholesteryl ester formation by macrophages. At the same time the lysosomal degradation of LDL in macrophages was inhibited under these conditions. These effects depended on the ganglioside structure and concentration. It is suggested that the effects observed could be caused by GM3-induced modification of LDL to a form that becomes recognized by macrophages.

One of the earliest events in the development of atherosclerosis is migration of monocytes from blood into the arterial wall, where they differentiate into macrophages and accumulate high amounts of cholesteryl esters and other lipids. These lipid-loaded cells are called foam cells. The lipids which accumulate in foam cells are believed to be derived from the plasma low density lipoproteins (LDL) inspite of the fact that native LDL* are weakly recognized by macrophages in vitro and that even high concentrations of native LDL when incubated with macrophages do not induce accumulation of much cholesteryl esters within the cells. It has been shown however that several artificial modifications of LDL can transform them into particles which are rapidly taken up by macrophages in vitro and cause them to accumulate massive amounts of cholesteryl esters (1). It is therefore believed that some type of LDL-modification in vivo occurs which transforms LDL into a form recognized by a high-affinity receptor on macrophages. However the nature of this modification remains still unknown.

The artificial modifications of LDL producing lipoprotein particles that can cause foam cell formation from cultured macrophages include acetylation

* The term "native LDL" refers to LDL isolated from normal human blood by sequental density ultracentrifugation according to standard methods.

Abbreviations: LDL-low density lipoproteins; GM3- II^3-NeuAc-Lac-Cer.

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(2), acetoacetylation (3) and conjugation with malonaldehyde (4) which hardly can be expected to occur in vivo. Another type of modification leading to enhanced LDL uptake by macrophages and more likely to take place in vivo is LDL-oxidation. This process has been studied extensively (reviewed in 1), however other biological pathways of LDL- modification, based on formation of complexes of LDL with components of the intracellular matrix or plasma, are not excluded. Among them, the modification of LDL by gangliosides is of special interest because gangliosides are normal components of human plasma (5) and aortal intercellular space (6) and are actively bound by LDL (7).

Recently we demonstrated that gangliosides in vitro modified size, stability and fluorescence properties of LDL as well as their interaction with antibodies against apo-B (7). Previously we (8) and others (9) found that preincubation of LDL with gangliosides reduced LDL binding to fibroblasts and hepatoma cells and LDL-uptake by these cells. In the present study we investigate the influence of GM3, the main ganglioside of human plasma, on the uptake and degradation of LDL by mouse peritoneal macrophages.

MATERIALS AND METHODS

Ganglioside GM3 was isolated from human liver (10). [^{14}C]Oleic acid 50-60 Ci/mmol was recieved from Amersham.

Human LDL (d=1.019-1.063 g/ml) were obtained from fresh plasma of normal volunteers by sequential ultracentrifugation as described in (11). [^{125}I]-LDL was prepared by the iodine monochloride method (12), specific activity was 50-100 cpm/ng protein. Association of gangliosides with LDL was achieved by incubation of LDL (2.5 uM) with 50 uM gangliosides for 0.5-2 h at 37^{o}C.

Mouse peritoneal macrophages were collected from unstimulated mice in 199 medium containing 20 units heparin/ml (13). Macrophages from 20 mice (5x10^{8} cells) were pooled and collected by low speed centrifugation at 1000 rpm for 5 min at room temperature. The cells were then resuspended in 199 medium containing 20% bovine serum, 100 ug/ ml of penicillin and 100 ug of streptomycin. Cells (1x10^{6} per well) were dispensed into 24-well tissue culture cluster (Costar, USA) with a density of 10^{6} cells/ well and allowed to adhere for 4-6 h at 37^{o}C in a humidified 5% CO_2/95% air atmosphere. Then the cells were washed twice with 199 medium to remove nonadherent cells. The 199 medium containing 20% human lipoprotein-deficient serum was added to each well after which the macrophages were used for experiments.

LDL binding and degradation were performed as described in (14). The macrophage monolayers were incubated in presence of [^{125}I]-LDL (100 ug protein/ml) or LDL-ganglioside associates at 37^{o}C for 3 h. The cell monolayers were washed three times with 199 medium containing 20% bovine serum albumine. The radioactivity associated with the cells was measured in a gamma-counter (LKB, Bromma, Sweden, model 1282). The proteolytic degradation of [^{125}I]-LDL and LDL-ganglioside associates was measured by assaying the amount of [^{125}I]-labeled trichloracetic acid-soluble material formed by the macrophages and excreted into the culture medium. For this aim the macrophage monolayers were incubated for 24 h as described for binding experiments. The medium removed after incubation was used to study LDL proteolytic degradation.

Incorporation of [^{14}C]-oleate by macrophages into cholesteryl esters and triglycerides was determined as follows (14). The macrophage monolayers were incubated with 0.3 ml 199 medium containing 10% of lipid-deficient serum, various amounts of native LDL or LDL-ganglioside associates and 0.2 umol/ml

[^{14}C]-oleic acid/albumine (M/M) at 37^{o} for 24 h. After incubation the monolayers were washed with PBS and the total lipids were extracted in situ with hexan/isopropyl alcohol (3:2). The cell residues were used to determine the cellular protein (15). The extracts of macrophage monolayers from three wells were combined and the cholesteryl esters and triglycerides were isolated by silica gel thin-layer chromatography using the solvent system hexan/ diethyl ether/ acetic acid (85:15:1 v/v). The cholesteryl ester and triglyceride bands were visualized with iodine vapor, scraped into vials and counted in a Racbeta 1250 beta-counter.

The incorporation of chlesterol by macrophages was determined as described early (14). The macrophage monolayers were incubated with 0.3 ml of 199 medium containing 20% human lipoprotein-deficient serum and several amounts of LDL-ganglioside assosiates or native LDL for 24 h at 37^{o}C. The cells were washed with PBS, and lipids were extracted as described above. The total cholesterol content in lipid extracts was determined using Boehringer Mannheim Monotest Cholesterol CHOD-PAP-Method (Cat. no 290 319, Boehringer, FRG).

RESULTS AND DISCUSSION

As it can be seen from the data of Table 1, the uptake of LDL by macrophages increases after preincubation of the lipoproteins with GM3. This effect of the ganglioside was concentration dependent and saturable at concentrations of GM3 exceeding $2x10^{-5}$ M. Further increase in the ganglioside concentration had no influence on the uptake of lipoproteins by the cells.

At the same time the lysosomal degradation of LDL in macrophages, as determined by the appearance of [^{125}I]-labeled products in the incubation medium, was inhibited in the presence of GM3. This effect depended again on the ganglioside concentration.

Pretreatment of LDL with GM3 appears to stimulate not only LDL-macrophage binding but also the uptake of lipoproteins by the cells as it could be inferred from the greatly enhanced intracellular cholesterol accumulation in presence of GM3 (Table 2). The presence of GM3 stimulated also LDL-induced formation of cholesteryl esters and triglycerides by macrophages in a

Table 1. Influence of ganglioside GM3 on the uptake and the degradation of LDL by macrophages

GM3 nmol/mg protein	^{125}I-LDL ng/ μg cell protein	
	bound	degraded
-	1.26±0.40	1.64±0.21
10	1.42±0,52	1.22±0.08
20	2.10±0.48	0.91±0.08

The results of 3 experiments.

Table 2. Influence of ganglioside GM3 on the LDL-induced accumulation of cholesterol in macrophages

Lipoproteins	Cholesterol (µg/ mg cell protein)
native LDL	18.35 ± 0.47
LDL+GM3	32.9 ± 0.94

concentration dependent manner (Table 3). These effects depended strongly on the chemical structure of the ganglioside: in contrast to GM3, ganglioside GD1a which is present in serum only as a minor ganglioside component, inhibited both LDL-induced intracellular cholesterol accumulation as well as cholesteryl ester and triglyceride formation by macrophages (data not shown).

The mechanisms underlying the influence of GM3 on LDL uptake and lipid accumulation by macrophages are unknown. The effects observed could be caused by interaction of the ganglioside with the recipient cells (with myelogenous leukemia cells GM3 induces macrophage-like cell differentiation (16) as well as by GM3-induced modification of LDL. At the moment we are not able to discuss the first possibility because of lack of evidence but we should like to consider some arguments in favor of the latter.

The major part of plasma gangliosides is associated with LDL (5). Recently we showed that LDL were able to bind relatevelly high amounts of gangliosides and that incubation of native LDL with gangliosides results in formation of LDL-ganglioside complexes which differ from native LDL in their physico-chemical properties such as: floating density, particle size, molecular organization of surface lipids and apolipopropteins, and immunoreactivity of apo-B (7). These changes seem to be caused partly by aggregation of LDl in presence of gangliosides. Brown and Goldstein (17) and Khoo et al (18) suggested that aggregated LDL might be active inductors of LDL-uptake by macrophages and enhence of cholesteryl ester accumulation in

Table 3. Influence of ganglioside GM3 on formation of cholesteryl esters and triglycerides in macrophages

Lipoproteins (µg protein/ml)	[^{14}C]-oleic acid content (pmoles/mg cell protein)	
	Cholesteryl esters	Triglycerides
native LDL (300)	0.03±0.01	3.65±0.83
LDL+GM3 (100)	0.15±0.01	10.13±0.20
LDL+GM3 (200)	0.14±0.07	11.05±0.80
LDL+GM3 (300)	0.22±0.07	29.34±2.73

these cells. On the other hand, we demonstrated that in the atherosclerotic arterial wall, especially in atherosclerotic lesions of the intima, the ganglioside content was at least three times higher than in unaffected regions and that atherosclerotic plaques were exceptionally rich in GM3 (6). Therefore it may well be that in such cases both LDL and arterial wall associated macrophages reside in a relativelly high ganglioside environment corresponding to the ganglioside concentration used in the presente work. Taking into account that fast growing cells actively release gangliosides into the medium (19) it seems to be possible that in damaged regions of the aorta LDL-ganglioside complexes are formed wich are rapidly taken up by phagocytes residing within the lesions, giving rise to formation of foam cells.

Such mechanism could also explain how arterial endothelial (20) and smooth muscle cells (21) modify LDL to a form that becomes recognized by macrophages. The ganglioside-induced modification of LDl seems to be essential but not sufficient for transformation of macrophages into foam cells. In any case, the idea that ganglioside-induced modification of LDL and/or the effects of ganglioside on macrophages associated with wessel wall may play a role in the pathogenesis of atherosclerosis, deserves further investigation.

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