Distribution of exogenously added gangliosides in serum proteins depends on the relative affinity of albumin and lipoproteins

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Abstract Gangliosides in normal serum are found only in lipoproteins and the relative content of the three major lipoprotein fractions is low density lipoprotein > high density lipoprotein > very low density lipoprotein (LDL > HDL > VLDL). Upon in vitro incubation of labeled gangliosides with human serum, about 15% of the exogenous gangliosides became associated with the albumin fraction and 85% were distributed on the lipoproteins in the order HDL > LDL > VLDL. To compare the relative affinities of serum proteins for gangliosides, the levels of exchange of exogenous gangliosides between preloaded serum proteins were determined. Although albumin had the highest binding capacity for gangliosides, 85% of the albumin-loaded gangliosides were transferred to the total lipoprotein fraction and this exchange was reversible. The transfer rate from albumin to isolated lipoproteins was higher to LDL (90%) and HDL (85%) whereas only 55% of albumin-loaded gangliosides were transferred to VLDL. The study of exchanges of preloaded gangliosides between isolated lipoproteins showed that the extent of transfer of gangliosides from a given lipoprotein fraction onto other lipoproteins was inversely correlated with its endogenous ganglioside content. Moreover, in the absence of albumin from the incubation medium, the final lipoprotein distribution of remaining exogenous gangliosides was similar to the normal distribution of endogenous gangliosides in serum lipoproteins. The formation of unexchangeable complexes between albumin and micellar exogenous gangliosides could be a possible explanation for the observed differences in the distribution of exogenous and endogenous gangliosides in serum proteins.—**Rebbaa, A., and J. Portoukalian.** Distribution of exogenously added gangliosides in serum proteins depends on the relative affinity of albumin and lipoproteins. *J. Lipid Res.* 1995. **36:** 564–572.

Supplementary key words gangliosides • VLDL • LDL • HDL • albumin

Gangliosides are glycosphingolipid constituents of the cellular plasma membrane. They are composed of a lipophilic residue consisting of an amide-linked long sphingoid base and a fatty acid that is believed to be localized in the outer leaflet of the plasma membrane and a hydrophilic carbohydrate moiety containing one or several molecules of sialic acid. Gangliosides are believed to play an important role in the interactions of cells with the extracellular microenvironment (1).

The gangliosides and neutral glycosphingolipids of normal human serum have been analyzed (2) and were found to be associated to the serum lipoproteins (3, 4). In patients with malignant tumors, gangliosides are thought to be released in significant amounts by rapidly proliferating cells, and the shed gangliosides modulate the activity of the immune system (5). The total ganglioside content of the serum has been shown to be modified in patients with head and neck tumors (6). Increased serum concentrations of GM3 and GD3 have been described in the serum of tumor-bearing melanoma patients (7). In neuroblastoma and retinoblastoma patients, GD2 was found to be increased in the serum (8, 9).

Clinical trials using injection of gangliosides have recently been designed to induce an immune response to cancer (10, 11) and to boost recovery after spinal cord injury (12). The design of such therapeutic protocols could be improved by a better understanding of the fate of the injected gangliosides. We recently found that, upon incubation of rat serum with labeled gangliosides, these gangliosides bind mostly to the high density lipoproteins (HDL), but also significantly to albumin, and a similar pattern was found in the serum of rats 3 h after intravenous injection of 200 μl autologous serum preincubated 2 h with 100 μg labeled gangliosides (13). However, although albumin was shown to bind gangliosides strongly when incubated in vitro (14, 15) this major serum protein appears to carry only trace amounts of gangliosides

Abbreviations: TCA, trichloroacetic acid; TLC, thin-layer chromatography; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LFF, lipoprotein-free fraction. Gangliosides are named according to the ganglioside nomenclature of Svennerholm (31) and the IUPAC-IUB recommendations (32). GM1, II^{3}Neu5AcGgose$_4$Cer, β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-Cer; GM3, II^{3}Neu5AcLcoseCer, α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-(1-1)-Cer.

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in normal serum (4) as well as in sera from cancer patients (16); in such normal and pathological sera, low density lipoproteins (LDL) contain the largest proportion of gangliosides. In order to determine the role of the major serum proteins that might be involved in the transport of exogenous gangliosides, the exchange of exogenously added gangliosides between the major serum proteins (albumin, VLDL, LDL, and HDL) was investigated. We report here the transfer of gangliosides between serum proteins after preincubation of isolated protein fractions with radiolabeled gangliosides.

MATERIAL AND METHODS

Separation of serum lipoproteins

Human AB serum was purchased from the Blood Transfusion Center of Lyon. The lipoprotein fractions were separated by density gradient ultracentrifugation (17) Chylomicrons were first extracted by centrifugation of serum at 10,000 rpm for 1 h. VLDL were floated at a density of 1.006 g/ml (density of serum). LDL and HDL were floated at respective densities of 1.063 and 1.21 g/ml. These densities were adjusted with solid potassium bromide. The amount of sodium bromide was calculated according to the following formula: M = (df – di)Vi/1 – (df.0.264); df, final density; di, initial density; Vi, initial volume. Each ultracentrifugation step was performed at 40,000 rpm for 24 h at 10°C in a Kontron TI 50 rotor. The floating lipoproteins were collected by suction with a Pasteur pipette, resuspended in a volume of PBS equivalent to the initial volume of total serum, and submitted to a second cycle of density gradient ultracentrifugation to obtain homogenous fractions. LDL and HDL were dialyzed against PBS buffer to remove potassium bromide. The homogenity of lipoprotein fractions was controlled by electrophoresis on polyacrylamide gradient. The protein content of each fraction was assayed by the method of Bradford (18).

Extraction of gangliosides

Gangliosides were extracted from the lipoprotein fractions as previously described (19). Three hundred ml of chloroform–methanol 1 2 (v/v) was added to 30 ml of each fraction. The mixture was sonicated for 3 min and then gently shaken for 12 h. The solution was filtered and the residue was resuspended in 150 ml of chloroform–methanol 2:1 (v/v), then shaken for 6 h. After a second filtration, the two filtrates were pooled and evaporated to dryness. The lipids were partitioned three times by centrifugation in chloroform–methanol–PBS 1:1:0.7 (by volume). To remove salts and contaminating molecules, the pooled aqueous phases containing the gangliosides were applied to a C18-bonded silica gel column (20). After washing with demineralized water, gangliosides were eluted from the column with methanol, chloroform–methanol 2:1 and concentrated. The assay of total lipid-bound sialic acid was performed using the periodate-resorcinol method (21). The neutral glycosphingolipids were purified from the lower phases of partition by the acetylation procedure of Saito and Hakomori (22), after removal of the remaining gangliosides by ion-exchange chromatography on DEAE-Sephadex A-25 (Pharmacia, Paris, France). Gangliosides and neutral glycosphingolipids purified from a known amount of each lipoprotein fraction were chromatographed on aluminium-backed silica gel 60 HPTLC plates (Merck, Darmstadt, Germany) in the following solvent systems: chloroform–methanol–0.2% aqueous calcium chloride 55:45:10 (by volume) for separation of gangliosides, and chloroform–methanol–water 65:25:4 for the neutral glycolipid fraction. After visualization of lipid spots on the plates with orcinol–sulfuric acid (1 min at 180°C in an oven), the stained lipids were quantitated by scanning densitometry at 540 nm with a CS-930 Chromatoscan (Shimadzu, Kyoto, Japan).

Exchanges of gangliosides between protein fractions

Ganglioside interaction with protein fractions was studied using radiolabeled GM3 and GM1 gangliosides. GM3 was purified from human melanoma tumors (11) and labeled to a specific activity of 9 μCi/μmol by selective N-acetylation with [^{14}C]acetic anhydride (Amersham, Paris, France) of N-deacetylated GM3 (23) purified by HPLC after treatment for 4 h at 80°C with KOH 0.1 M in 90% n-butanol (24). GM1 from calf brain was labeled to a final specific radioactivity of 41 μCi/μmole by catalytic tritiation of the double bond of sphingosine with potassium borotritide on palladium acetate (25). [^{14}C]GM3 (2.5 μg) was incubated with 0.5 ml of the various serum protein fractions to determine their ganglioside binding capacity. After gradient density centrifugation to separate the proteins from the supernatant, the protein-associated radioactivity was counted with an MR 300 beta scintillation counter (Kontron, Paris, France). To study the transfer of gangliosides from albumin to lipoproteins, 2 μg [^{14}C]GM3 was incubated for 15 min at 37°C in 0.5 ml PBS containing 0.5 mg albumin. Then, 1.5 ml PBS containing VLDL (0.5 mg), LDL (1.75 mg), HDL (2.75 mg), or 1.5 ml of human normal serum was added. After 1 h incubation at 37°C, the medium was diluted to 6 ml with PBS containing the adequate amount of potassium bromide and the lipoproteins were separated from albumin by density gradient ultracentrifugation. Fractions of 1 ml were collected and the radioactivity was counted in each. The transfer of gangliosides from lipoproteins to albumin was carried out as described above after loading the lipoproteins with 2 μg [^{14}C]GM3. The transfer of [^{3}H]GM1 between lipoprotein fractions was determined by preincubation of 0.5 ml of a lipoprotein fraction with

2 μg [^{3}H]GM1 at 37°C for 15 min. Then, 0.5 ml each of the two other lipoprotein fractions was added to reach the relative ratio of the lipoproteins in serum. The lipoproteins were incubated together for 1 h at 37°C, then separated by gradient density centrifugation and the radioactivity was counted in each fraction.

Preparation of liposomes containing gangliosides and interaction with lipoproteins

Pig brain sphingomyelin, cholesterol, and [^{14}C]GM3 (9 μCi/mg) were mixed at molar ratios of 1:1:0.1. In order to monitor the binding of the liposomal lipids, [^{3}H]cholesterol and [^{14}C]phosphatidylcholine (Amersham, Les Ulis, France) were used with unlabeled GM3 in some experiments. The lipids were taken up in ethanol, injected into PBS and sonicated for 5 min to obtain small unilamellar liposomes. The interaction of liposomal gangliosides with isolated lipoproteins was studied by incubating (37°C) 80 μl containing 120 μg VLDL, 280 μg LDL, or 440 μg HDL with 80 μl of the liposome solution containing 1.6 μg [^{14}C]GM3. The solution was then centrifuged for 5 min at 50,000 *g* in an Air Driven Ultracentrifuge (Beckman, Paris, France), giving a pellet of liposomes and a supernatant containing lipoproteins. The radioactivity was counted in both fractions with a beta scintillation counter MR 300 (Kontron, Paris, France).

RESULTS

Distribution of gangliosides in the major protein fractions of normal serum

Serum proteins were separated by sequential ultracentrifugation into three fractions containing, respectively, VLDL, LDL, and HDL, and a fourth fraction devoid of lipoproteins (lipoprotein-free fraction, LFF). The four fractions were then analyzed by thin-layer chromatography for their ganglioside and neutral glycolipid contents. The total amount as well as the distribution of gangliosides and neutral glycolipids differed among these four fractions. As reported previously by others (3, 4, 16), LDL contained the largest amount of gangliosides (54% of total serum gangliosides), followed by HDL (34%) and VLDL (12%). LDL also contained the largest proportion of neutral glycolipids (72%), followed by HDL (20%) and VLDL (5%). LFF contained trace amounts of gangliosides and no detectable neutral glycolipids.

Interaction of radiolabeled gangliosides with the protein fractions of the serum

The total serum was incubated for 2 h with [^{14}C]GM3; the proteins were precipitated with TCA 10% and centrifuged for 5 min at 4°C. Ninety-five percent of the added radioactivity was found in the pellet, indicating that the exogenous GM3 was associated with the serum proteins.

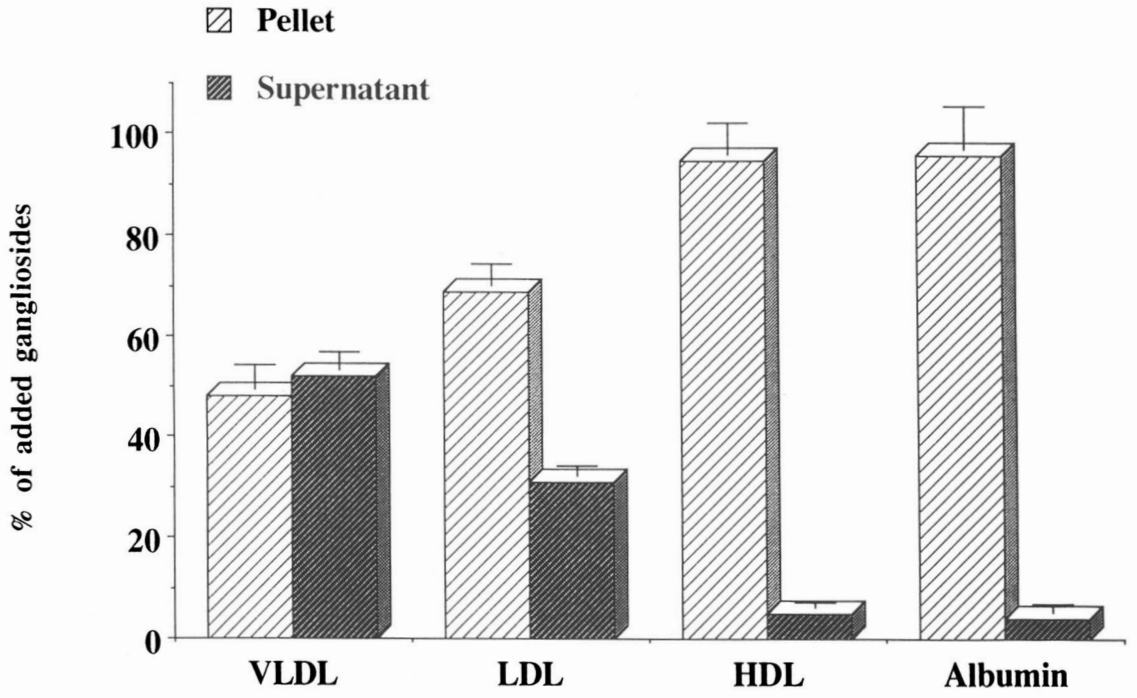

Fig. 1. Binding of [^{14}C]GM3 to lipoproteins and albumin in vitro. [^{14}C]GM3 (2.5 μg) was incubated at 37°C with each of the lipoprotein fractions at physiological concentrations (150 μg VLDL, 350 μg LDL, 550 μg HDL in a total volume of 100 μl PBS), or with 550 μg albumin. Proteins were then precipitated at 4°C with 300 μl 10% TCA and centrifuged at 4°C for 5 min at 10,000 *g*. Radioactivity was measured in the pellet and the supernatant. Clear bars, pellet; dark bars, supernatant. Values are expressed as dpm ± SEM of three separate experiments.

In separate experiments with unlabeled GM3, the supernatant and the resuspended pellet were neutralized with 2 N NaOH. GM3 was recovered by reverse-phase chromatography on C18-bonded silica gel; no product of degradation of GM3 was detectable by TLC.

Binding of gangliosides to serum proteins was at a maximal level within 5 min for the tested proteins (data not shown) and the incubation times used in our study were considered to give equilibrium conditions. The interaction of exogenous GM3 with purified serum protein fractions was determined by incubating the labeled ganglioside with isolated lipoprotein and albumin fractions for 2 h before precipitation with 10% TCA. The lipoprotein fractions were used at physiological concentrations (1.5 mg/ml for VLDL, 3.5 mg/ml for LDL, and 5.5 mg/ml for HDL), whereas albumin was used at the same concentration as HDL (5.5 mg/ml) which is tenfold lower than the physiological level. As shown in **Fig. 1,** HDL bound more gangliosides than LDL and VLDL, thus confirming our previous finding that exogenously added gangliosides become preferentially associated to HDL (13). The binding capacity of isolated albumin toward [^{14}C]GM3 was found to be very high, in accordance with previous reports (14, 26). In separate experiments, ultracentrifugation with gradients of potassium bromide was used to recover the serum proteins incubated with labeled gangliosides and the values found for binding of gangliosides to the various proteins were consistent with the data obtained by precipitation of proteins with 10% TCA.

Upon incubation of liposomal gangliosides with VLDL or LDL followed by ultracentrifugation, only a few percent of the gangliosides were not found in the liposomal pellet, showing a very slow rate of transfer from small unilamellar vesicles to VLDL and LDL, even after 15 h incubation (**Fig. 2**). Incubation of liposomes containing GM3 with HDL resulted in a large amount of the ganglioside becoming associated to the lipoprotein. However, using liposomes made with unlabeled GM3, [^{3}H]cholesterol, and [^{14}C]phosphatidylcholine, all radioactive lipids were recovered in the HDL fraction, suggesting that the transfer of lipids from liposomes to HDL occurred at a high rate for all liposomal lipids. This has already been documented in previous reports (26, 27) showing a reversible transfer of glycosphingolipids between liposomes and HDL_3.

Transfer of radiolabeled gangliosides between ganglioside-loaded proteins and the other serum proteins

As only trace amounts of gangliosides can be detected in serum albumin, the comparative affinity of lipoproteins and albumin for gangliosides was investigated. The binding of gangliosides to serum proteins was maximal after 15 min (not shown) and this time of incubation was considered to be sufficient to reach equilibrium. To determine whether the gangliosides are transferred from albumin to the lipoproteins, albumin was preincubated with low amounts of [^{14}C]GM3 or [^{3}H]GM1, then added to a sam-

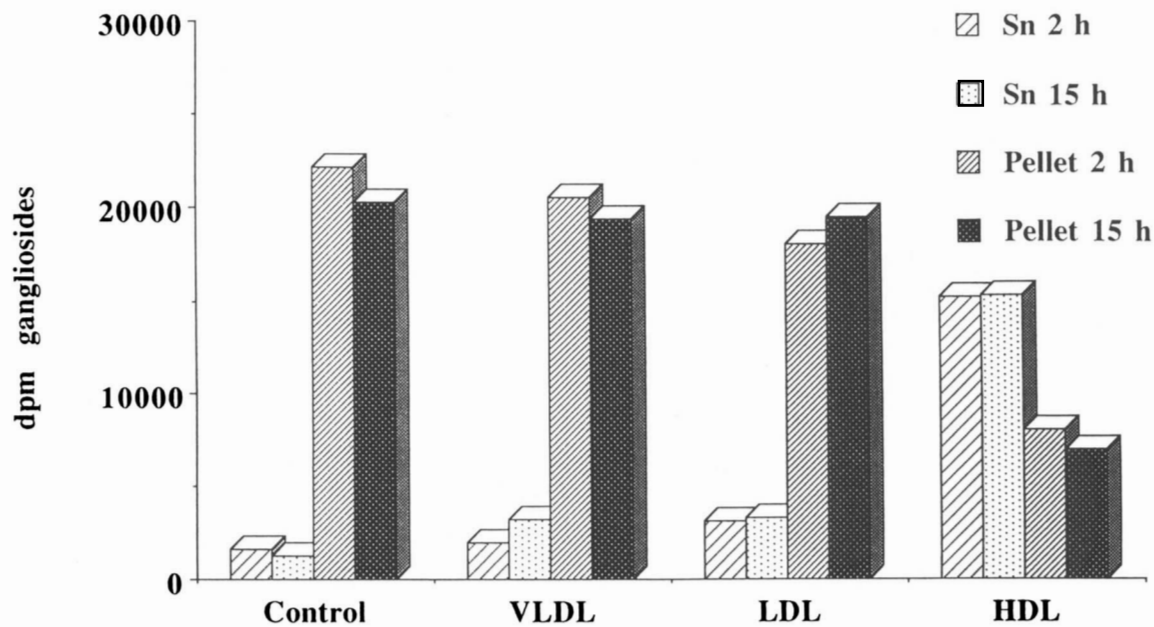

Fig. 2. Transfer of [^{14}C]GM3 from liposomes to isolated lipoprotein fractions. After incubation of liposomes with VLDL, LDL, or HDL, and ultracentrifugation for 30 min at 50,000 *g*, radioactivity was measured in the liposome-containing pellet and the supernatant containing the lipoproteins. Sn 2h and Sn 15h are the supernatants after separate incubations for 2 and 15 h. Pellet 2h and Pellet 15h are the corresponding pellets. The control was incubation with PBS alone.

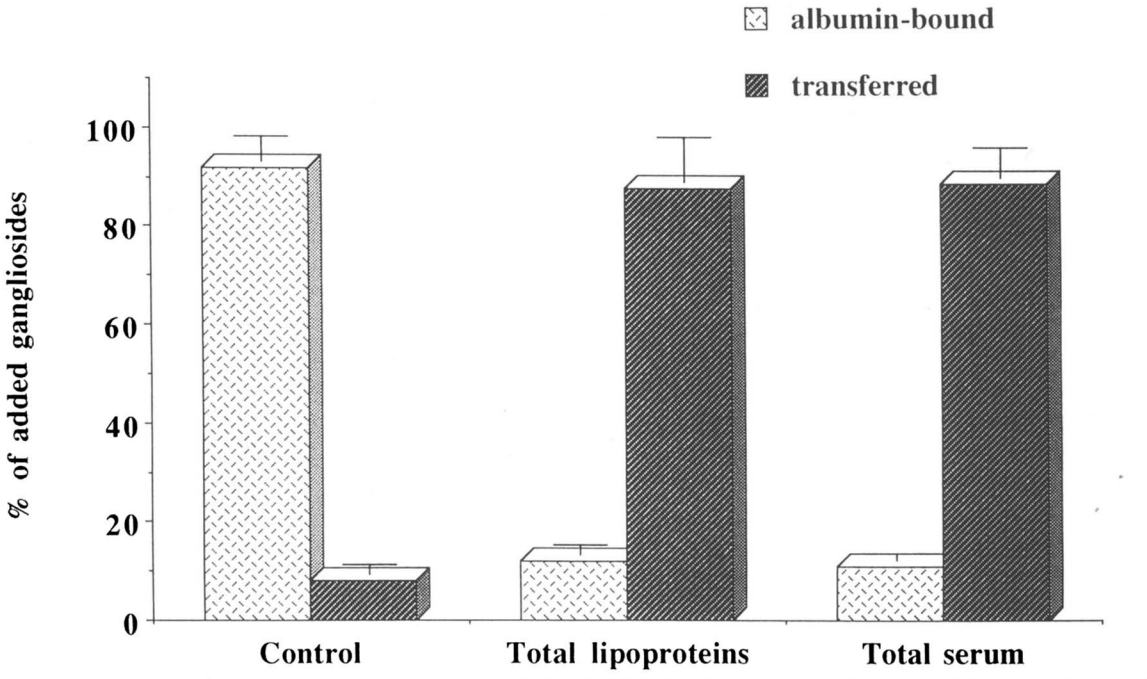

Fig. 3. Transfer of albumin-loaded [^{14}C]GM3 to the serum lipoprotein fraction. Five hundred μg of albumin in 0.5 ml PBS was preincubated 15 min at 37°C with 2 μg [^{14}C]GM3. Then, 1.5 ml PBS containing either one of the lipoprotein fractions VLDL (0.5 mg), LDL (1.75 mg), HDL (2.75 mg), or 1.5 ml of human normal serum was added. After 1 h incubation at 37°C, lipoproteins were separated from albumin by density gradient ultracentrifugation and radioactivity was counted in each fraction. Dashed bars, % gangliosides remaining on albumin; hatched bars, % transferred gangliosides ± SEM of three separate experiments. The controls represent the proportions of gangliosides remaining on albumin recovered by density gradient ultracentrifugation before and after incubation with lipoproteins.

ple of total serum or to isolated lipoprotein fractions. After separation by ultracentrifugation in 6-ml tubes and collection of 1-ml fractions, the radioactivity was present only in the top fraction containing the lipoproteins and in the bottom fraction containing albumin. The latter fraction was precipitated with 10% TCA in separate experiments and no free labeled ganglioside was detectable in the supernatant, suggesting that the ganglioside was indeed associated with the protein. As can be seen in **Fig. 3,** most of the albumin-loaded gangliosides was transferred

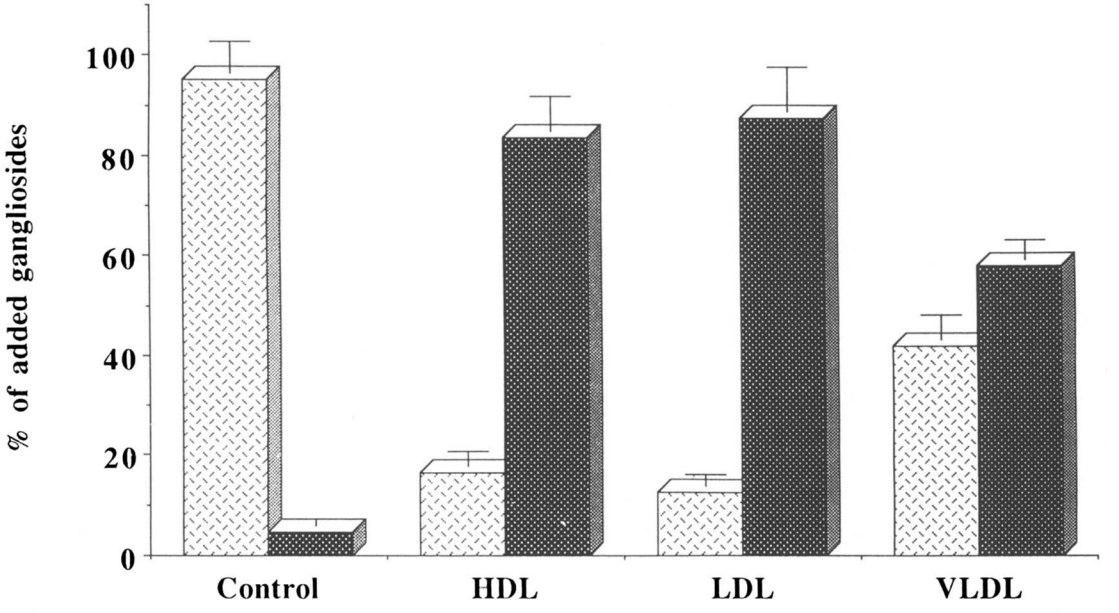

Fig. 4. Transfer of [^{3}H]GM1 from albumin to each class of lipoprotein. Two μg [^{3}H]GM1 was incubated with 0.5 mg albumin in 500 μl PBS for 15 min at 37°C. Then 500 μl PBS containing either 0.5 mg VLDL, or 1.75 mg LDL, or 2.75 mg HDL was added. After 1 h incubation at 37°C, albumin was separated from the lipoproteins by gradient density ultracentrifugation and radioactivity was counted in each fraction. Dashed bars, % gangliosides remaining on albumin; dotted bars, % transferred gangliosides ± SEM of three separate experiments. Controls are as in Fig. 3.

at equilibrium to the lipoprotein fraction of the serum, while a significant part (15%) was reproducibly found to remain associated to albumin. Experiments with ganglioside-loaded lipoproteins and ganglioside-free albumin showed that the exchange was reversible and gave the same 15% proportion of gangliosides on albumin (not shown). When albumin and isolated lipoprotein fractions were used, the extent of transfer was quite different with regard to each lipoprotein. **Figure 4** shows that, although HDL and LDL took up 80–90% of the albumin-loaded gangliosides, only 55% was transferred to VLDL which seems to be the lipoprotein fraction with the lowest affinity for gangliosides. In experiments of incubation of ganglioside-loaded isolated lipoproteins with albumin, the proportions of gangliosides remaining associated with each lipoprotein after coincubation suggested that the transfer of gangliosides was also reversible in the same proportions (not shown). Varying the time of ganglioside loading onto proteins from 15 min to 3 h did not change the extent of transfer (not shown).

The exchange of gangliosides between lipoprotein fractions was studied by loading each lipoprotein (100 μg) with 1 μg [^{3}H]GM1 prior to incubation with the two other fractions. The respective amounts of the three major lipoprotein fractions used for these experiments reflected their relative serum concentrations. All fractions were then isolated by sequential ultracentrifugation. As shown in **Fig. 5,** more than 70% of the VLDL-loaded GM1 was transferred in equal amounts to HDL and LDL. A large proportion of the HDL-loaded GM1 was exchanged with the other lipoproteins, mostly with LDL, whereas only one fourth of the LDL-associated GM1 could be found on VLDL and HDL upon incubation. It is noteworthy that the proportions of exogenous gangliosides remaining on each of the three lipoprotein fractions after in vitro experiments of transfer of loaded gangliosides between isolated lipoproteins are directly correlated with the relative amounts of native gangliosides carried by the lipoproteins (**Fig. 6**). However, this holds true for lipoproteins only when albumin is absent from the incubation medium, whereas in the presence of albumin, the results give an actual distribution of gangliosides in lipoproteins that is the one shown in Fig. 1.

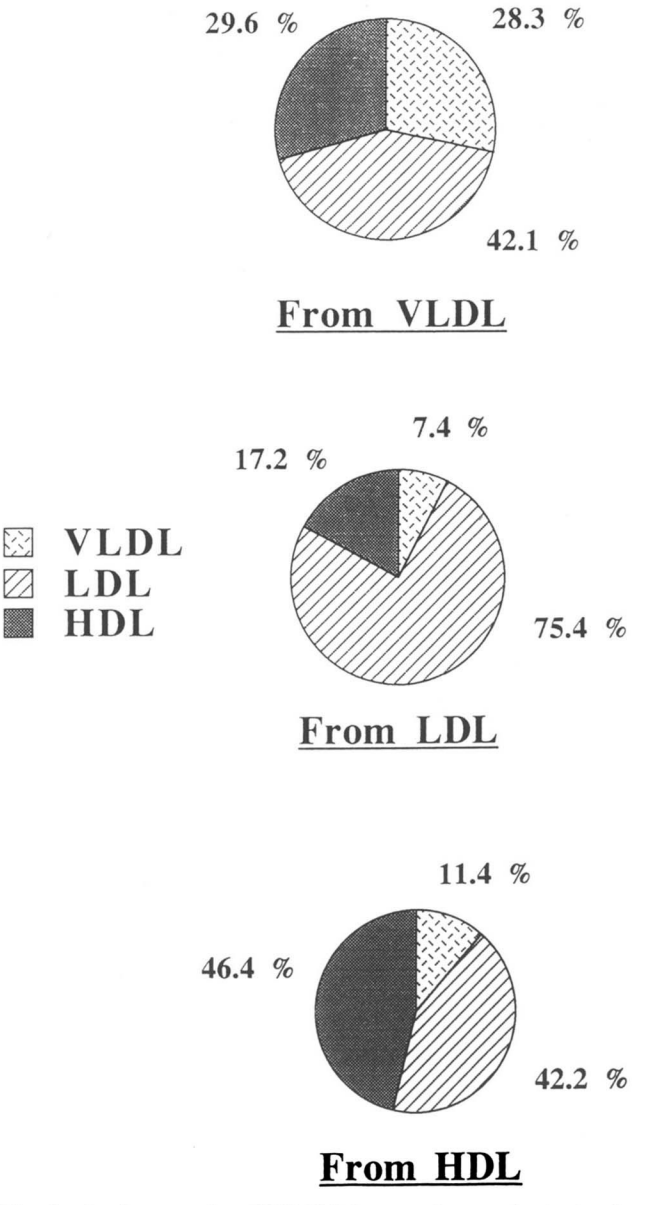

Fig. 5. In vitro transfer of [^{3}H]GM1 between lipoproteins in the absence of albumin. Two μg [^{3}H]GM1 was incubated for 15 min at 37°C in 500 μl PBS containing the amount of each lipoprotein purified from 0.5 ml of serum, then the two other lipoprotein fractions were added to reach the known relative ratio of lipoproteins in serum. After 1 h at 37°C, the lipoproteins were separated by gradient density ultracentrifugation and the radioactivity was counted in each fraction. Data give the percent distribution of preloaded gangliosides associated with each fraction after the transfer experiments. Variations between three experiments were below 5%.

DISCUSSION

The present study shows that serum gangliosides are found only in the lipoproteins and predominantly in the LDL fraction, in accordance with previous investigations (3, 4, 16). The lipoprotein-free fraction, containing mostly albumin with some high molecular weight proteins, yielded only trace amounts of gangliosides. However, we observed earlier that after in vitro and in vivo incubation of serum with exogenous gangliosides, a significant amount (in the 15–20% range) became associated with the albumin fraction, whereas the distribution of gangliosides among lipoproteins suggested a higher binding to HDL (13). In the present study, incubation of radiolabeled GM3 with isolated albumin and lipoprotein fractions revealed that albumin has the same high capacity as lipoproteins to bind gangliosides, and the kinetics of gan-

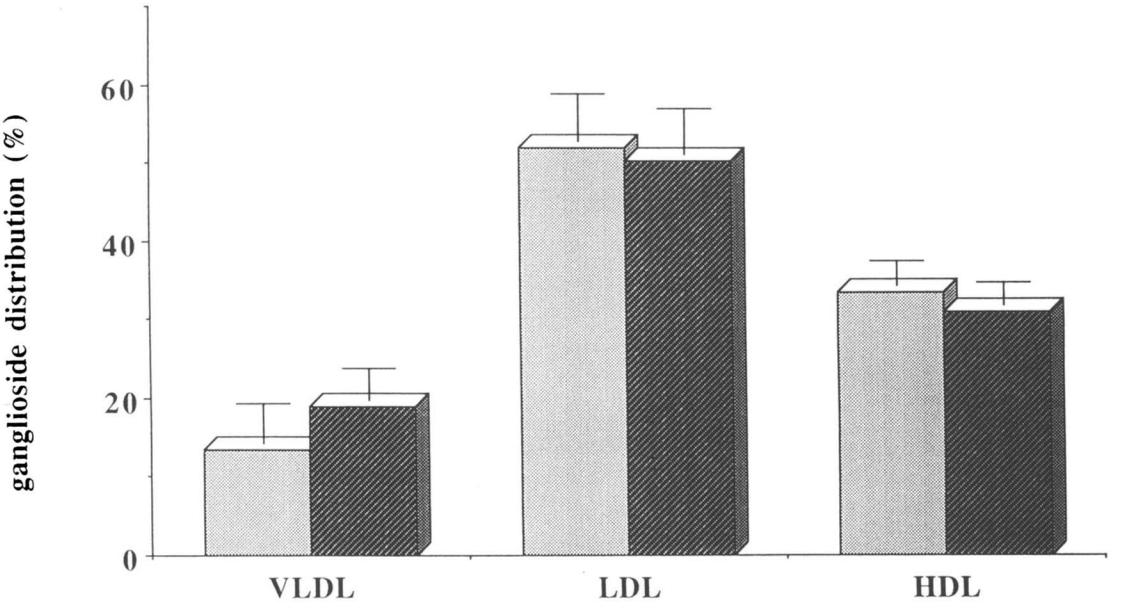

Fig. 6. Distribution of gangliosides in serum lipoproteins. Dotted bars, % native gnagliosides; hatched bars, relative % of loaded gangliosides remaining in lipoproteins fractions after in vitro transfer experiments between lipoproteins in the absence of albumin. The line above each bar represents SEM of three separate experiments.

glioside binding are similar to those observed for HDL. The transfer of gangliosides from liposomes to serum proteins occurred with a very low yield, except with HDL which bound 70% of the liposomal gangliosides and also the same proportion of the liposomal phospholipids and cholesterol. Such a high rate of transfer has been reported by Kwok, Shen, and Dawson (27) and Shen, Kwok, and Dawson (28) who showed that the exchange of lipids was reversible; but the binding of micellar glycolipids to HDL[3] was considered by these authors as an artifact due to a similarity in the flotation density of micellar glycolipid aggregates and HDL[3] since these authors found that glycolipids and HDL incubated together have then a different elution rate upon gel filtration chromatography. This explanation obviously does not account for the binding of gangliosides to VLDL and LDL, and in the present study gangliosides were still associated to proteins after precipitation with 10% TCA which cannot precipitate micellar gangliosides, suggesting that the simultaneous recovery of gangliosides and proteins after density gradient ultracentrifugation is not merely coincidental.

The results obtained with the transfer experiments of exogenous gangliosides between albumin and a total lipoprotein fraction indicate that lipoproteins have a greater affinity for gangliosides than albumin in vitro. Up to 90% of the gangliosides associated with albumin are taken up by HDL and LDL, but only 55% are transferred to isolated VLDL. Nevertheless, loading either albumin or lipoprotein fraction with gangliosides, the same significant proportion (15–20%) of gangliosides remains associated to albumin upon incubation with lipoproteins. Therefore, the transfer of gangliosides between serum proteins is reversible and probably depends upon the relative affinities of each protein for gangliosides. As to the occurrence of the albumin-associated gangliosides observed in the present study, this binding might result from the micellar state of gangliosides in the incubation medium. In the 1 to 5 μg/ml concentration range that was used in our experiments, GM1 and GM3 are in a micellar form and therefore may begin to form stable complexes with albumin during the 15-min incubation (14, 24) precluding further transfer of the complexed gangliosides to lipoproteins. The recovery of 15% of injected gangliosides in the albumin fraction of rat serum after intravenous injection of the autologous serum preincubated 2 h with gangliosides (13) is also consistent with the observed occurrence of such ganglioside–albumin complexes upon incubation of GM1 with total serum (15). Thus, albumin interferes in the interaction of these exogenously added gangliosides with lipoproteins, whereas there is no such influence of albumin in the protein binding of tumor-shed gangliosides that were shown to be distributed in the serum proteins of tumor-bearing patients as the native gangliosides in normal serum (16). Although the mechanism of shedding from tumor cells is still unclear, preliminary experimental evidence suggest that gangliosides are shed as monomeric molecules (A. Rebbaa and J. Portoukalian, unpublished results). Tomasi et al. (14) observed that the binding of GM1 to albumin is much slower at submicellar concentrations of ganglioside. Moreover, the interaction was reported to be reversible and did not lead to the formation of albumin–ganglioside complexes.

Therefore, it is possible that monomeric molecules of gangliosides would not bind albumin. If confirmed, this postulated physical state of tumor-shed gangliosides would favor their interaction with lipoproteins that have a greater affinity than albumin for gangliosides.

Unlike albumin which binds gangliosides through an hydrophobic bond with the ceramide moiety (14), the interaction of gangliosides with lipoproteins also involves the apolipoprotein as Ledvinova, Iwamori, and Nagai (29) reported that apoB interacts with the carbohydrate moiety of glycosphingolipids. This additional interaction is likely to account for the higher affinity of lipoproteins for gangliosides. The present study shows that high proportions of preloaded gangliosides are rapidly transferred between lipoproteins. When albumin is absent from the incubation medium, the distribution of the remaining percentage of gangliosides on the lipoproteins after transfer experiments between isolated lipoproteins is strikingly similar to that of the native gangliosides associated with these fractions. In the presence of albumin, the lipoprotein distribution of gangliosides is similar to that obtained when the gangliosides are either injected in vivo or incubated in vitro in a total serum.

The use of gangliosides injected as therapeutic agents to patients in clinical trials (10–12) raises many questions about their fate and biological effects that may be critically dependent on the serum proteins to which they are associated. We very recently showed that the in vitro immunomodulatory effect of melanoma gangliosides is much stronger with gangliosides loaded to VLDL, and a less potent effect was seen with LDL, whereas HDL and albumin had no influence (30). These results suggest that the carrier protein is of importance regarding the biological effect of gangliosides. Studies of the influence of carrier proteins on the in vivo biological effect of gangliosides are currently in progress in our laboratory.

This study was supported by grants from Caisse Nationale d'Assurance Maladie (C.N.A.M.), Fédération des Groupements des Entreprises Francaises pour la Lutte contre le Cancer (F.E.G.E.F.L.U.C.), and Association pour la Recherche sur le Cancer.

Manuscript received 31 May 1994 and in revised form 28 September 1994.

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