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Cholesterol Homeostasis in Rat Astrocytoma Cells GA-1[1]

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Astrocytes play a key role in cholesterol metabolism in central nervous system. We have shown that fetal rat astrocytes in primary culture secrete cholesterol-rich HDL with the endogenous apolipoprotein (apo) E and generate cholesterol-poor HDL with exogenous apoE and apoA-I [Ito *et al.* (1999) *J. Neurochem.* 72, 2362]. In order to study these reactions in relation to the stage of cell differentiation, we examined generation of HDL by rat astrocytoma cells. Lack of apoE secretion was found in three astrocytoma cell lines, human T98G, rat C6, and GA-1 [Kano-Tanaka *et al.* (1986) *Proc. Jpn. Acad. Ser. B* 62, 109]. GA-1 produced apoE at very low level and therefore generated much less HDL by itself than the astrocytes in primary culture. In contrast, GA-1 interacted with exogenous apoE and apoA-I to produce cholesterol-rich HDL while the astrocytes produced cholesterol-poor HDL with these apolipoproteins. Cholesterol biosynthesis rate measured from mevalonate was higher and down-regulated more by LDL in the astrocytes than GA-1. On the other hand, the cellular cholesterol level, uptake of LDL, and cyclodextrin-mediated non-specific diffusion of cholesterol from cell surface were same between these two cells. Treatment of GA-1 with acidic fibroblast growth factor influenced neither the production of apoE nor the baseline lipid secretion, but increased the cholesterol synthesis from mevalonate and the magnitude of its down-regulation by LDL, and decreased cholesterol content in the HDL produced by exogenous apoA-I. In conclusion, suppression of apoE biosynthesis in the undifferentiated astrocytes GA-1 resulted in poor secretion of cholesterol-rich HDL and in turn more production of HDL with exogenous apolipoprotein. Cellular cholesterol homeostasis was altered accordingly.

Key words: apoE, astrocyte, astrocytoma, cholesterol, HDL.

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The central nervous system (CNS) of the vertebrates is segregated by the blood-brain barrier (BBB) from the systemic circulation, and a regular extracellular lipid transport system by plasma lipoprotein is not generally available to the cells in this organ. Hence, extracellular lipid transport in CNS is carried out by an independent intra-CNS lipoprotein system that is mostly high density lipoprotein (HDL) containing apolipoprotein (apo) E synthesized mainly by astrocytes (*1–4*) and in part by microglias (*5*, *6*), or apoA-I from unknown sources (*7–9*). The astrocytes are known to excrete apoE with cellular cholesterol and phospholipid in a form of cholesterol-rich HDL as a main HDL in CNS (*2*, *4*, *10–12*). The secretion of apoE and HDL by the various astrocytoma cells was also demonstrated in the brain tissues (*13–15*) and in culture (*15–17*).

Many interesting findings implicated the roles of apoE in pathological stages of CNS. ApoE accumulates in the damaged lesion of central and peripheral nerve tissues (*18–25*). An isoform of apoE, apoE4, is strongly correlated to the risk of Alzheimer's disease (*26–30*). Many "apoE receptors" are identified in the brain as members of the low density lipoprotein (LDL) receptor superfamily (*31*, *32*). Thus, the apoE, HDL, and their relating system in CNS seem very essential for cholesterol homeostasis and damage-recovery of the brain. We may therefore postulate that astrocytes play a central role in this system by synthesizing apoE and generating HDL.

We have characterized the HDL assembly in rat fetal astrocytes with endogenous apoE and exogenous apolipoproteins (*33*, *34*). In agreement with the previous other works, astrocytes synthesized apoE and secreted cholesterol-rich HDL. In addition, the cells interacted with exogenous apoA-I, A-II, and E to generate HDL. Unlike many other cells reported by ourselves and by other groups (*35*, *36*), the HDL thus generated by astrocytes contained less cholesterol than the HDL secreted with the endogenous apoE (*33*). This restricted cholesterol availability was released by the pretreatment of the cell with sphingomyelinase, suggesting that the HDL assembly by exogenous apolipoprotein takes place at the sphingomyelin/cholesterol rich domain of the plasma membrane (*34*).

In order to study further the HDL assembly by astro-

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Abbreviations: CNS, central nervous system; BBB, blood brain barrier; HDL, high density lipoprotein; apo, apolipoprotein; LDL, low density lipoprotein; aFGF, acidic fibroblast growth factor; PBS, 10 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl; BSA, bovine serum albumin; TLC, thin layer chromatography; SDS, sodium dodecyl sulfate; ACAT, acylCoA:cholesterol acyltransferase.

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cytes, we undertook the study of the astrocytes on an undifferentiated stage since at least one of the human glioma cell lines had been shown not to produce apoE (*15*). Astrocytoma cell line GA-1 that was previously established in this laboratory by transforming the rat astrocytes *in vitro* by the treatment with epoxy resin–polyamine components (*37–40*) was characterized for the cellular cholesterol homeostasis in relation to the apoE-HDL production and the reactivity to the exogenous apolipoprotein in comparison to the rat astrocytes in primary culture. The apoE-HDL production was suppressed while the reactivity to the exogenous apolipoprotein was higher in the undifferentiated stage of the cells.

MATERIALS AND METHODS

Cell Cultures—Rat astrocytoma cells, GA-1, was previously established from the transformed rat astrocytes in culture by chemical adhesive of epoxy resin–polyamine components (*37*). Rat astrocytoma cells C6, human malignant astrocytoma cells T98G, and human macrophage cell line cells THP-1 were all obtained from American Type Cell Culture. Astrocytes were prepared from the brain of day 17 Wistar rat fetus according to the previous method (*41*, *42*). All cells were grown in the F-10 medium containing 10% fetal calf serum at 37°C in an atmosphere with 5% CO_2. In order to induce the differentiation of GA-1, the cells were treated with acidic fibroblast growth factor (aFGF) (Santa Cruz Biotechnology, CA) at 100 ng/ml for 3 days. THP-1 cells were treated with phorbol 12-myristate, 13-acetate for 48 h to induce the expression of the apoE gene (*43*).

Lipoproteins and Apolipoproteins—HDL and LDL were isolated by ultracentrifugation from fresh human plasma in the density ranges 1.063–1.21 and 1.006–1.063 g/ml, respectively. LDL was labeled with 1α,2α(*n*)-^{3}H cholesteryl oleate (Amersham) according to the method previously described (*35*, *44*). ApoA-I was isolated from human HDL by delipidation and anion-exchange chromatography as previously reported (*45*). Recombinant human apoE3 was a generous gift by Mitsubishi Tokyo Pharmaceutical Ind. (Yokohama).

Cellular Lipid Release—Cellular lipid release from the astrocytes and GA-1 was measured according to the method previously described (*33*). Rat GA-1 and astrocytes at the confluent stage were washed with 10 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl (PBS) four times and then cultured in the F-10 medium containing 0.02% bovine serum albumin (BSA) for 24 h. The cells were then incubated in the fresh medium for 24 h with LDL containing [^{3}H]cholesteryl ester (25 μg of protein) and 0.5 μCi of (methyl-^{3}H)choline chloride (NEN, MA). After washing, further incubation in F-10 medium containing 0.02% BSA for 24 h and washing, apolipoproteins or 2-hydroxypropyl-β-cyclodextrin (*46*) were added to the cell culture medium containing 0.02% BSA. The medium was recovered after the incubation for certain periods of time and centrifuged to remove cell debris. Lipid was extracted from a 500-μl aliquot of the medium and from the cells as described previously (*33*). The radioactivity of cholesterol, cholesteryl ester, phosphatidylcholine, and sphingomyelin was counted after separation by thin layer chromatography (TLC). Cellular lipids were measured by enzymatic colorimetric assay method for cholesterol and choline-phospholipid (T-chol C-test and Phospholipid B-test, respectively, from Wako Pure Chemicals, Tokyo). Specific radioactivity of each cellular lipid was calculated and used for estimation of the lipid mass released in the medium. Cellular lipid release was measured also after the pretreatment of GA-1 with aFGF. All the assays were done in triplicate and the results were expressed as mean ± SE.

Density Gradient Analysis of the Medium—The culture media after the 24-h incubation with apolipoproteins were analyzed by ultracentrifugation with sucrose density gradient between 1.006 and 1.2 g/ml (*33*, *35*). The medium, 8 ml, was centrifuged at 10,000 rpm for 10 min to remove cell debris, and a 7-ml aliquot was overlayered on the layers of the solutions of 1.20 g/ml (8-ml) and of 1.07 g/ml (8-ml). After centrifugation at 49,000 rpm for 48 h in a Hitachi P50AT2 rotor, the solution was recovered in 12 fractions from the bottom of the tube (1.9 ml each). Radioactivity was counted in cholesterol and phosphatidylcholine after the lipid was extracted and separated by TLC for each fraction.

Immunoblot Analysis—Rat astrocytoma cells GA-1 and C6, rat fetal astrocytes, human astrocytoma cells T98G and human macrophage cell line cells THP-1 were analyzed for the synthesis of apoE. The cells were washed with PBS four times and cultured in the F-10 medium containing 0.02% BSA for 24 h on confluent stage. THP-1 cells were differentiated by phorbol 12-myristate, 13-acetate for 48 h (*43*). The cells were incubated in fresh medium for 24 h with and without LDL (25 μg protein), washed and further incubated in the F-10 medium containing 0.02% BSA for 24 h. A 500 μl aliquot of each medium was centrifuged to remove cell debris and protein was precipitated in 10% trichloroacetic acid solution. After centrifugation at 15,000 rpm for 10 min, the pellet was redissolved in 50 μl of 10% sodium dodecyl sulfate (SDS) solution, sonicated, and boiled for 5 min. A 10 μl aliquot was analyzed by electrophoresis in a 10% polyacrylamide gel with 1% SDS in Tris-glycine buffer. Electrophoretic Western blot transfer onto a 0.2-μm nitrocellulose membrane was performed at 4°C in Tris-glycine buffer for 3 h at 55–60 V, and subsequent blocking and washing steps were performed using Tris-buffered saline, pH 7.4, containing 0.05% Tween 20 and containing 5% skim milk powder. Following the transfer, the membrane was blocked, washed and then incubated for 1.5 h with anti–rat apoE rabbit antiserum (1:500 dilution) generously provided by Dr. Jean Vance, The University of Alberta, Canada, or with anti–human apoE rabbit antibody purchased from Calbiochem-Novabiochem (CA). After washing the first antibody, the membrane was incubated with the second antibody (anti–rabbit IgG) conjugated with horseradish peroxidase (Biosource International) in 1:5,000 dilution. The washed membrane was incubated with ECL Western blotting detection reagent and exposed to high performance chemiluminescence film.

Reverse Transcript Polymerase Chain Reaction (RT-PCR)—Messenger RNA level of apoE was quantitated by RT-PCR. Total RNA fraction was isolated from GA-1 and the astrocytes in primary culture by acid guanidium thiocyanate–phenol-chloroform extraction, reverse-transcribed to cDNA using a Super Script Preamplification System (Gibco BRL), and the resulting cDNA was subjected to PCR by using the antisense DNA probe 5′-GCGCACCTCCTCCAT-CTGCTC-3′ and the sense probe 5′-AGGATCTACGCAAC-

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CGACTCG-3′. After the electrophoresis of the product, agarose gel was stained with freshly prepared SYBR® Gold nucleic acid gel stain solution. The bands were detected by a UV transilluminator, UVP NLM-20E, at the wavelength 302 nm.

Intracellular Cholesterol Esterification—To probe an intracellular cholesterol compartment available for esterification by acylCoA:cholesterol acyltransferase(ACAT), incorporation of [^{14}C]oleic acid (0.5 μCi) (Amersham) into cholesteryl ester was determined (*47, 48*). After incubation of the cells with apolipoprotein for 1–2 h and, the cells were incubated with [^{14}C]oleic acid for 2 h and incorporation of the radioactivity into cholesteryl ester fraction was measured. The assay was triplicated the results were presented as mean ± SE.

Cholesterol Synthesis and Its Down Regulation—Lipid synthesis from acetic acid was found extremely low in GA-1 indicating the presence of metabolic defect in acetylCoA biosynthesis, so that the cellular cholesterol synthesis was examined for the steps after HMG-CoA reductase that include farnesyl diphosphate synthase and squalene synthase, by labeling the cells with mevalonolactone, RS-(5-^{3}H) (American Radiolabeled Chemicals). The cells, rat GA-1 and astrocytes, were washed with PBS four times and cultured in the F-10 medium containing with 0.02% BSA for 24 h on a confluent stage. Various amounts of LDL were incubated with the cell for another 24 h. The cells were washed with PBS four times and incubated in the F-10 containing BSA with [^{3}H]mevalonolactone for 2 h. The cells were washed four times with cold PBS and the cellular lipid was extracted with chloroform/methanol (2:1 v/v). Free cholesterol radioactivity was determined after separation by TLC. The same experiment was also performed with the GA-1 pretreated with aFGF. The assay was triplicated the results were presented as mean ± SE.

RESULTS

Secretion of apoE was examined for the rat astrocytes and rat astrocytoma cell lines GA-1 and C6, as well as human astrocytoma T98G. Figure 1 shows secretion of apoE by the astrocytes and by GA-1 and C6 with and without cholesterol loading from LDL, and also by T98G in comparison to the differentiated human macrophage cell line THP-1. ApoE in the medium was visualized by immunoblotting. While the astrocytes and THP-1 cells are shown to secrete substantial amount of apoE, no detectable amount of apoE was demonstrated in the medium of GA-1, C6, and T98G even after preincubation with LDL. The mRNA was however detectable in GA-1 by RT-PCR through the signal was weak (Fig. 1C). Hence, GA-1 has been characterized for cholesterol release and homeostasis as a representative astrocytoma in comparison to the astrocytes.

Figure 2 shows the uptake of LDL by GA-1 and astrocytes. Cholesterol content was approximately same for these two types of cells for the baseline and for its increase by incubating with LDL. Indeed, the uptake of the radio-

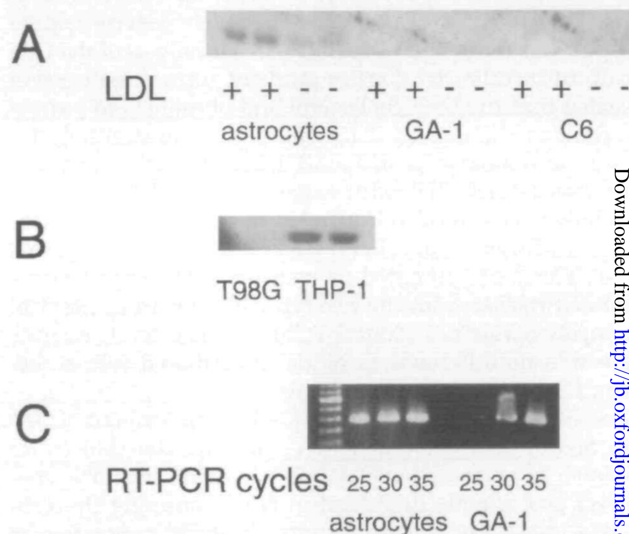

Fig. 1. **Synthesis and secretion of apoE from the astrocytomas.** A, rat astrocytes, GA-1 and C6 were loaded with of LDL (25 μg protein) for 24 h and the culture medium was analyzed by immunoblotting as described in the text by using anti–rat apo-E rabbit antiserum. Cell protein per well was 286 ± 15 and 301 ± 12 μg for the astrocytes with and without LDL, 750 ± 59 and 706 ± 26 for GA-1, and 591 ± 60 and 543 ± 46 for C6, respectively. B, human astrocytoma T98G, and human macrophage cell line cells THP-1 pretreated with phorbol 12-myristate, 13-acetate were also analyzed for apoE secretion by using anti-human apoE antibody. Cell protein per well was 788 ± 24 and 768 ± 46 μg for T98G and THP-1, respectively. C, RT-PCR analysis of the apoE mRNA of the astrocytes and GA-1 without loading LDL, as described in the text starting with total RNA 5 μg.

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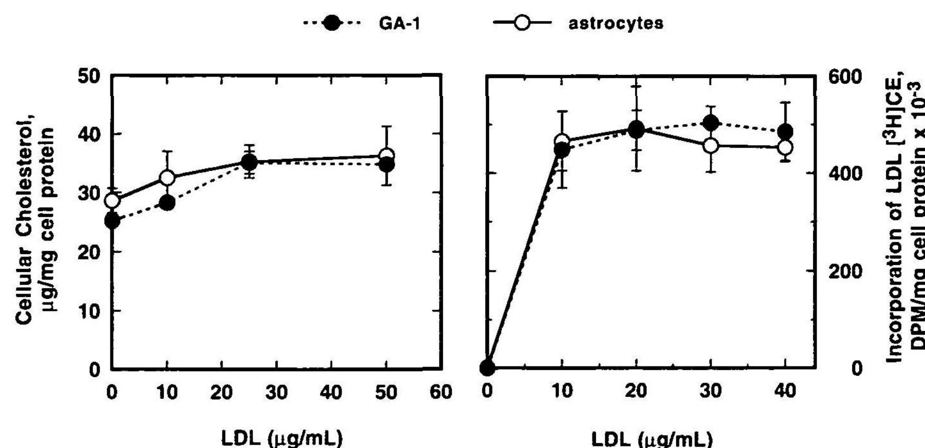

Fig. 2. **Uptake of cholesteryl ester by rat astrocyte in primary culture (open circles) and by rat astrocytoma GA-1 (closed circles).** The cells were prepared in 3-cm culture plates. Various amounts of LDL (left panel) and ^{3}H-cholesteryl ester labeled-LDL (right panel) were added to the culture medium. After incubation of the cells at 37°C for 24 h, the cellular cholesterol was measured by using enzymatic colorimetric assay kits (left) and the radioactivity in the cell was determined by a liquid scintillation counter (right). Cell protein was 310 ± 70 μg per well for the astrocytes and 471 ± 52 μg per well for GA-1.

labeled cholesteryl ester from LDL was identical between these cells in a dose-dependent manner. Figure 3 demonstrates the release of cellular cholesterol and phospholipid to the medium when GA-1 and astrocytes were preloaded for 24 h with LDL (25 μg of protein/ml of medium). Cholesterol release by GA-1 was approximately 1/4 of that by astrocytes in the absence of apoA-I. When apoA-I is present in the medium, cholesterol and phospholipid were released from GA-1 in a dose dependent manner. In contrast, astrocytes release cholesterol poorly by apoA-I being consistent with our previous finding (*33*). Figure 4 shows the release of cholesterol, phosphatidylcholine and sphingomyelin from GA-1 by apoA-I and apoE. The results again demonstrated that all the lipids were released by either apolipoproteins in a dose dependent manner. Interestingly, cholesterol release by apoE was lower than that by apoA-I. Analysis of the GA-1 culture medium by density gradient ultracentrifugation revealed that the both cholesterol and phospholipid formed the peak at the density 1.15 g/ml and it substantially increased in the presence of apoA-I, indicating the generation of cholesterol-rich HDL with exogenous apoA-I (Fig. 5).

Cellular cholesterol release also takes place by its non-specific diffusion from cellular surface through the aqueous phase. This was estimated by measuring the cyclodextrin-mediated release from the two types of cells. In contrast to the apolipoprotein-mediated cellular cholesterol release, there was no difference in diffusion mediated release between GA-1 and astrocytes (Fig. 6).

Response of intracellular cholesterol compartment of GA-1 to apoA-I was examined by estimating the cholesterol available for the reaction of ACAT. The ACAT available cholesterol pool rapidly decreased in GA-1 reflecting the substantial release of cholesterol into the HDL generated by apoA-I (Fig. 7).

Figure 8 shows cholesterol biosynthesis from mevalonate and its down regulation by extracellular LDL. The rate of cholesterol synthesis measured for the steps after HMG-CoA reductase is three times higher in the astrocytes than GA-1 and down-regulated to the same level by LDL.

In order to examine the effect of cytokines to induce differentiation of GA-1 on the parameters for cellular cholesterol homeostasis described above, the cells were treated with aFGF. ApoE secretion was not detected by immunoblot even after the aFGF treatment (data not shown) and apoE mRNA expression was not changed by aFGF (Fig. 9). For the apoA-I–mediated cell lipid removal, the pretreat-

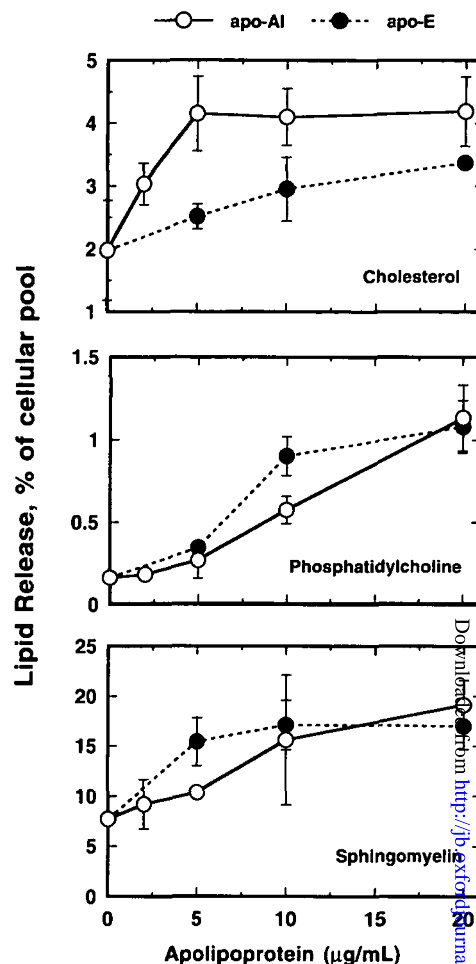

Fig. 4. **Release of cholesterol, phosphatidylcholine, and sphingomyelin by rat GA-1 cells.** The cells were loaded and labeled with ^{3}H-cholesteryl ester-labeled LDL (25 μg of protein/ml of medium) and also labeled with 0.5 μCi of ^{3}H-choline chloride for 24 h as described in the text. After the cells were washed, various amounts of human apoA-I (open circles) and apoE (closed circles) were added to the 1 ml of cell culture in medium and incubated for 24 h. Lipid was extracted from the medium and cells with chloroform/methanol (2:1 v/v) and separated by TLC to determine the radioactivity of cholesterol, phosphatidylcholine, and sphingomyelin. Cell protein was 503 ± 72 μg/well. The results are presented as percentage of the respective cellular lipid based on the radioactivity.

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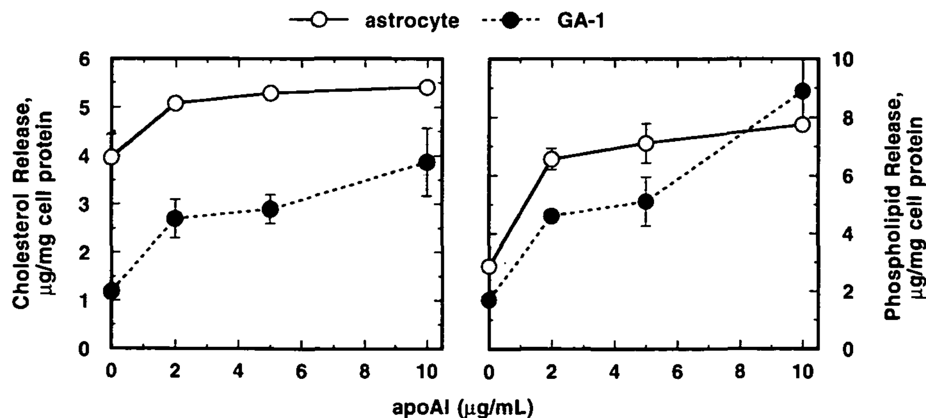

Fig. 3. **Release of cholesterol and phospholipid by the cells.** Rat astrocytes (open circles) and GA-1 (closed circles) were incubated with cold LDL (25 μg of protein/ml of medium) for 24 h in the 3-cm plates. After the cells were washed, various amounts of human apoA-I was added to the 1 ml cell culture medium, and incubated for 24 h. The medium lipid was extracted and separated by TLC as described in the text. Cholesterol and phospholipid was determined by using enzymatic colorimetric assay kits. Cell protein was 193 ± 22 and 667 ± 48 μg/well for the astrocytes and GA-1, respectively.

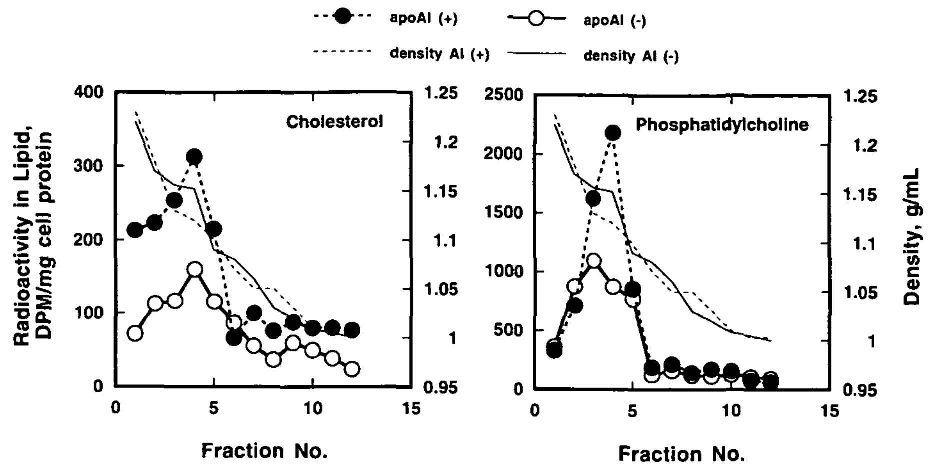

Fig. 5. **Density gradient ultracentrifugation analysis of the culture medium of rat GA-1 after incubation with apoA-I.** The cells were incubated in a 10-cm culture plate with and without apoA-I (50 μg/10 ml of medium), and the medium was analyzed by sucrose density gradient ultracentrifugation as described in the text. Radioactivity in cholesterol (left panel) and phosphatidylcholine (right panel) was determined for each of 12 fraction from the bottom to the top of the centrifuge tube. Open circles indicate the medium without apoA-I and closed circles with A-I. Solid lines indicate the density of each fraction without apoA-I and broken lines indicate that with apoA-I. Cell protein was 8.15 mg/plate without apoA-I and 7.21 mg/plate with apoA-I. Radioactivity in the cell cholesterol was 5,655 and 5,434 dpm/mg cell protein, without and with apoA-I, respectively, and that in the cell phosphatidylcholine was 10,413 and 9,987 dpm/mg cell protein without and with apoA-I.

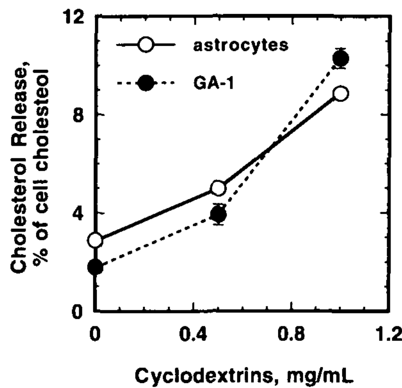

Fig. 6. **Release of cholesterol by cyclodextrin from rat astrocyte (open circles) and rat GA-1 cells (closed circles).** The cholesterol-labeled cells were incubated with the indicated amount of cyclodextrin for 24 h. The medium lipid was extracted with chloroform/methanol (2:1 v/v) and its radioactivity was determined by a liquid scintillation counter. Cell protein was 313 ± 19 and 443 ± 25 μg/well for the astrocytes and GA-1, respectively. The results are presented as percentage of the cellular cholesterol based on the radioactivity.

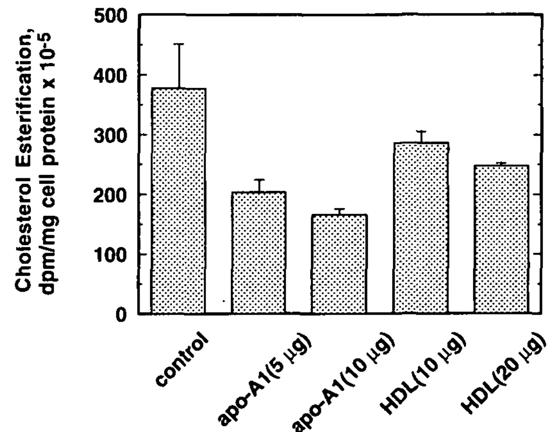

Fig. 7. **Incorporation of [^{14}C]oleate into cholesteryl ester by rat GA-1.** The cells in the 3-cm plates were loaded with LDL and then incubated with apoA-I (5 and 10 μg) and HDL (10 and 20 μg as protein) for 2 h. After washing, the cells was incubated with [^{14}C]oleate for 1 h and incorporation of the radioactivity into cholesteryl ester was determined. Cell protein was 404 ± 24 μg/well.

ment with aFGF selectively reduced cholesterol release, resulting in changing the HDL generated from cholesterol-rich to cholesterol poor (Fig. 10). For the cholesterol biosynthesis pathway from mevalonate and its down-regulation by LDL, the aFGF treatment increased the baseline rate and it was down-regulated to the same level as the untreated cells by LDL (Fig. 11). Thus, the differentiation of the GA-1 by aFGF transformed the cells toward the matured astrocytes with respect to these cholesterol homeostasis parameters.

DISCUSSION

GA-1 is a glioma cell line cell that is transformed from the rat fetal astrocytes *in vitro* by an unknown component(s) of chemical adhesive of epoxy resin–polyamine (*37*). The cells were previously demonstrated for chromosome abnormalities of 2q–, 4q+, and trisomy 12, and distribution patterns of silver-stained nuclear organizer regions (*38, 39*). GA-1 has also been characterized for the lack of response to glia maturation factor (*49*) and for growth inhibition by sialosyl cholesterol (*40*). As the lack of apoE secretion was demonstrated in the three astrocytoma cell lines including GA-1, this cell line was further characterized for cholesterol homeostasis as a representative undifferentiated astrocytes.

The glioma cells poorly secrete HDL by themselves reflecting the lack of apoE synthesis while the reactivity of the glioma to exogenous apolipoprotein was reciprocally higher to generate cholesterol-rich HDL. These findings are in contrast to the astrocytes in primary culture which secrete substantial amount of cholesterol-rich HDL with endogenously synthesized apoE and generate cholesterol-poor HDL by exogenous apolipoprotein (*33, 34*).

The attempt to measure cholesterol biosynthesis from acetate failed since the uptake of the radio-labeled acetate was almost negligible in GA-1, indicating the defect of ace-

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tylCoA biosynthesis. Cholesterol synthesis was therefore measured for the steps after HMG-CoA reductase by using radio-labeled mevalonate. In these steps, farnesyl diphosphate synthase and squalene synthase are known for regulation by the sterol regulatory element binding protein–mediated mechanism (*50*, *51*), so that the rate thus determined can be a limited parameter for regulation of cholesterol biosynthesis by a cellular cholesterol compartment.

Baseline cholesterol biosynthesis thus defined is set lower in GA-1 than the astrocytes though cellular cholesterol level was not apparently different, presumably in part by the lower secretion rate of cholesterol as the apoE-HDL. The uptake of LDL and the down-regulated level of the cholesterol synthesis rate by LDL are same between the astrocytes and GA-1 and may support the view that secretion of the cholesterol-rich apoE-HDL is a main cause of the difference in cholesterol homeostasis. However, the treatment of GA-1 with aFGF induced the higher cholesterol synthesis in GA-1 without apparent induction of apoE biosynthesis, indicating an independent effect of the aFGF-induced differentiation. Cholesterol release to cyclodextrin was not different between GA-1 and the astrocytes (Fig. 6). This parameter may probe a cholesterol compartment in cellular surface readily available for diffusion through the aqueous phase, and therefore distribution of cholesterol in this compartment is essentially unchanged by the transformation.

Apolipoproteins found in CNS are mainly apoA-I and apoE associated with HDL (*10*, *11*, *52*). The apoE in CNS is produced within CNS and unexchangeable with the apoE associated with plasma lipoprotein since it remained the original phenotype of the recipient even after the liver transplantation that results in the change of the plasma apoE phenotype to that of the donor (*53*). ApoE and apoA-I are found accumulated in the damaged lesions of CNS or peripheral nerve so that these apolipoproteins are thought to function in the healing process of the nervous tissues (*18–25*). ApoE in CNS has become more highlighted since one of its isoform, apoE4, has been identified as a risk factor for Alzheimer's disease (*26–30*) and for the deposition of amyloid β protein after head injury (*54*, *55*) or intracranial hemorrhage (*56*). Nevertheless, the mechanism underlying these clinical, epidemiological and pathological findings remain largely inconclusive in spite of the substantial accumulation of the experimental data in the past several years.

ApoE in CNS synthesized by astrocytes (*1–4*) and microglia (*5*, *6*) is secreted as prebeta-HDL–like lipoprotein particles with the cellular lipid (*2*, *4*, *11*, *12*). On the other hand, the "receptor" proteins that recognize lipid-bound apoE for the uptake have also been identified in CNS. Among those are the low density lipoprotein (LDL) receptor in the glias and neurons (*10*, *57*), the LDL receptor–related protein (*58*, *59*) and other "apoE receptors" (*31*, *32*). These findings implicate that apoE functions as a major extracellular lipid transporter in CNS. However, the true physiological ligands for many of these "receptors" remain unknown. It has recently been reported that the simultaneous disrup-

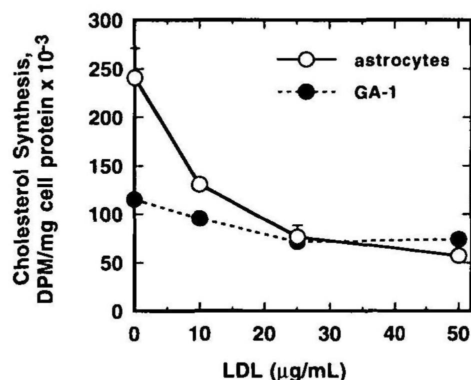

Fig. 8. **Cholesterol biosynthesis and its down-regulation by LDL in rat astrocyte (open circles) and in rat GA-1 (closed circles).** The cells were loaded with various amounts of LDL for 24 h. After washing, the cells were incubated with [^{3}H]mevalonolactone for 2 h. Lipid was extracted from cell and separated by TLC to determine the radioactivity of cholesterol. Cell protein was 207 ± 16 and 420 ± 61 μg/well for the astrocytes and GA-1, respectively.

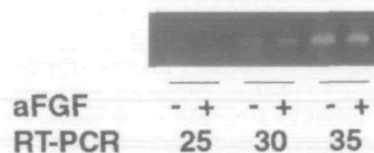

Fig. 9. **The effect of aFGF on the expression of apoE gene by the analysis of apoE mRNA in GA-1.** ApoE mRNA was quantitated by RT-PCR from total RNA 5 μg as described in the text. Cell protein was 443 ± 50 and 451 ± 62 μg/well for GA-1 without and with the aFGF treatment.

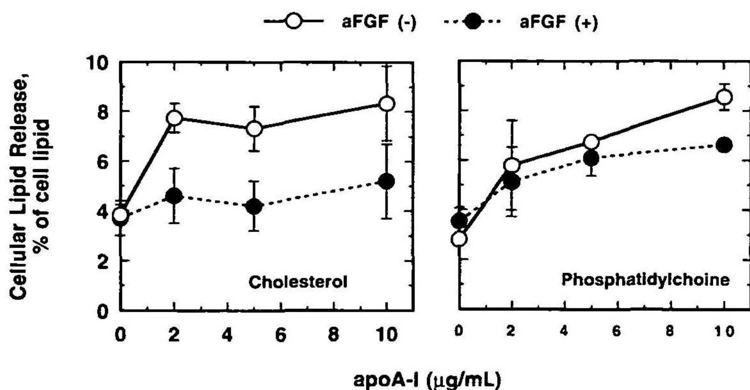

Fig. 10. **The effect of aFGF on the apoA-I–mediated cellular lipid removal.** Rat astrocytoma GA-1 cells were incubated with ^{3}H-cholesteryl ester–labeled LDL (25 μg of protein/ml of medium) and 0.5 μCi/ml of ^{3}H-choline chloride for 24 h. After washing, GA-1 were incubated with or without aFGF (100 ng/ml) for 24 h. The cells were washed with the buffer, followed by the incubation with aFGF again in the presence of various concentration of apoA-I for 24 h. The lipid was extracted from the cultured medium with chloroform/methanol (2:1 v/v). Free cholesterol and phospholipid were separated by TLC and its radioactivity was determined by a liquid scintillation counter. The results are presented as percentage of the respective cellular lipid based on the radioactivity. Cell protein was 557 ± 57 and 562 ± 67 μg/well for the control and aFGF treated cells, respectively.

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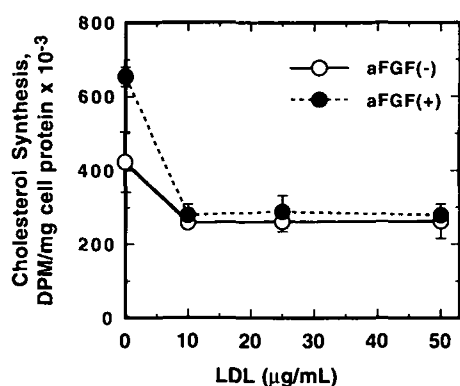

Fig. 11. **The effect of aFGF on cholesterol biosynthesis and its down-regulation by LDL.** Rat astrocytoma GA-1 cells were incubated with or without aFGF (100 ng/ml) for 3 days. After washing, the cells were incubated with various amounts of LDL for 24 h and labeled with 5 μCi/ml of ^{3}H-mevalonolactone for 2 h. The cellular lipid was extracted with chloroform/methanol (2:1 v/v). Free cholesterol was separated by TLC and its radioactivity was determined by liquid scintillation counter. Cell protein was 498 ± 82 and 437 ± 64 μg/well for the control and aFGF treated cells.

tion of two of these genes, very low density lipoprotein receptor and apoE receptor 2, in mouse causes severe developmental problem in cerebellum that resembles Reeler/Disabled (*60*). This finding implicates that these receptors function as essential signal mediators for cell migration but not as an endocytotic pathway for the ligands like other receptors in the same family.

Function of apoE is thus one of the keys to understand extracellular cholesterol transport in CNS, as well as its role in pathogenesis of Alzheimer's disease and the healing process of the CNS damage. In this paper, we have demonstrated that the astrocytoma cells synthesize much less apoE than the mature astrocytes and therefore hardly secrete HDL particle. Other parameters of cellular cholesterol homeostasis were also found altered in the astrocytoma. This seems primarily due to the immature stage of the cells since many of the nature of the matured cells are induced by the treatment of GA-1 cells with aFGF.

The results showed that highly undifferentiated astrocytoma may lack apoE synthesis and subsequent HDL secretion. This may not be so with more differentiated cells since many authors previously reported the synthesis of apoE by astrocytomas in tissues (*13–15*) and in culture (*15–17*). More clinical studies are required to establish if the apoE synthesis can be used a marker for the grade of differentiation of astrocytoma cells. If the synthesis of apoE and secretion of cholesterol-rich HDL are related to the brain function of the recovery from the damage, the astrocytoma cells may lack in such function by this impairment. This may leads to aggravation of the brain damage in the patients with undifferentiated astrocytoma.

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