Lipid composition of hepatitis B virus surface antigen particles and the particle-producing human hepatoma cell lines

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Abstract More than 90% of lipids of hepatitis B virus surface antigen (HBsAg) particles produced by two human hepatoma cell lines (huGK-14 and PLC/PRF/5) were composed of phospholipids, with phosphatidylcholine being the dominant component, accounting for more than 80% of total membrane lipids. Analysis of subclass compositions of phospholipids of HBsAg particles and the host cell lines revealed that 1,2-diacyl glycerophosphocholine was preferentially incorporated into the membrane of the HBsAg particles, although both host cell lines contained extremely high concentrations (more than 60% of total phospholipids) of ether-linked phospholipids. Phospholipids of other hepatoma cell lines (HuH-7, Hep-G2, and huL-1) which were not associated with hepatitis B virus (HBV) infection, were composed mostly of 1,2-diacylglycerophospholipids. Activities of dihydroxyacetone-phosphate acyltransferase, which is known to be an obligatory enzyme in ether lipid biosynthesis, were found to be elevated by three- to fourfold in both huGK-14 and PLC/PRF/5 cells compared to those of other hepatoma cell lines. The results suggest a possible relationship between HBV-induced hepatocellular carcinogenesis and the drastic change in the metabolism of membrane phospholipids. —**Satoh, O., M. Umeda, H. Imai, H. Tunoo, and K. Inoue.** Lipid composition of hepatitis B virus surface antigen particles and the particle-producing human hepatoma cell lines. *J. Lipid Res.* 1990. **31:** 1293–1300.

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Hepatitis B virus is a small DNA virus that causes acute and chronic liver disease and is also involved in the etiology of hepatocellular carcinomas (1, 2). During the chronic carrier state or the acute phase of the virus infection, large quantities of hepatitis B surface antigen (HBsAg) are secreted into the bloodstream. Electron microscopic analysis of the serum from hepatitis virus-infected individuals revealed that the HBsAg is associated with three types of particles, 22-nm spherical particles, tubular structures of about 22 nm in diameter with variable length, and 42-nm spherical Dane particles (3). Dane particles are the infectious virions and are composed of an nucleocapsid surrounded by a lipid-containing envelope with HBsAg. Of these particles, 22-nm spherical particles represent the bulk of HBsAg in the serum and are composed only of the HBsAg-containing lipid envelopes (4).

Although knowledge about the structure of the virus DNA, RNA, and gene products has increased in recent years, the details of the structure of the viral envelope and the mechanisms of assembly of the viral components are still poorly understood. A variety of animal viruses contain lipids as a major structural component of the virus particles. The study of the comparative lipid analyses of paramyxoviruses, myxoviruses, rhabdoviruses, and togaviruses showed that the composition of the viral lipids generally resembled the lipid composition of its host cell plasma membrane (5–7). Ben-Porat and Kaplan (8) showed that the phospholipid composition of herpesvirus was identical to that observed with the inner nuclear membrane from which it had budded. On the basis of these investigations it is generally accepted that compositions of the membrane where viruses bud rather than the affinity of the viral proteins to lipids determine the viral lipid composition (6, 9). Regarding the lipid composition of HBsAg particles, Gavilanes, Gonzalez-Ros, and Peterson (10) reported the outline of the lipid composition of the HBsAg particles obtained from human plasma. Since exchange and transfer as well as remodeling of lipids of viral particles may occur after the secretion into the circulation (11), the composition of the particles recovered from pa-

Abbreviations: HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; SDS, sodium dodecyl sulfate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; PS, phosphatidylserine; CL, cardiolipin; L-PC, lysophosphatidylcholine; TG, triglyceride; CH, cholesterol; CHE, cholesterol ester; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; TLC, thin-layer chromatography; FCS, fetal calf serum.

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tient serum may not be indicative of that of the nascent particles. The human hepatoma cell line, huGK-14 which could effectively produce HBsAg particles in a serum-free medium, was recently established (12). In the present study, we have undertaken the analysis of lipid components of the nascent HBsAg particles produced by huGK-14 cells and by another human hepatoma cell line, PLC/PRF/5 cells (13).

Ether-linked lipids are ubiquitously found in both normal and cancerous mammalian cells and tissues (14). Phospholipids of hepatocytes of many species contain exceptionally small amounts of either ether or vinylether type glycerophospholipids (15). Although many neoplastic tissues or cells had higher levels of ether-linked glycerophospholipids, the extent of the increase was within several percent of the phospholipids, in the observations so far reported (14, 16–18). During the course of experiments we found that both huGK-14 and PLC/PRF/5 cells had extremely high concentrations of ether-linked glycerophospholipids compared to those of normal human hepatocytes (15). Since the detailed analysis of phospholipid compositions of human hepatoma cell lines has not been done, we have undertaken an analysis of lipids of several human hepatoma cell lines. The relationship between the HBV-induced carcinogenesis and the altered metabolism of membrane lipids will be discussed.

METHODS

Cell lines and culture conditions

Human hepatoma cell line huGK-14 cells (12) were cultured in DM-160 medium (Kyokuto Pharmaceutical Industrial Co.) supplemented with 10% fetal calf serum. HuH-7 cells (19) were cultured in RPMI-1640 medium (Gibco) supplemented with 10% fetal calf serum. Hep-G2 (20), huL-1 (21), and PLC/PRF/5 cells (13) were cultured in Dulbecco's modified Eagle's medium (Flow Laboratories) supplemented with 10% fetal calf serum. Cells were grown in a humidified atmosphere of 5% CO_2 at 30°C. For the production of HBsAg particles from huGK-14 cells, the culture medium of the confluent layer of the cells was removed and was changed to Williams' E medium supplemented with 10^{-6} M dexamethasone, where fetal calf serum was not included (12). For the production of HBsAg particles from PLC/PRF/5 cells, the culture medium was replaced by Dulbecco's modified Eagle's medium supplemented with 1% of fetal calf serum. With a medium change of every 6 days, both huGK-14 and PLC/PRF/5 cells continuously produce HBsAg particles.

Preparation of HBsAg particles

HBsAg particles were purified from the culture medium of either huGK-14 cells or PLC/PRF/5 cells as described previously (12). Briefly, culture medium was concentrated by press filtration with a membrane filter (molecular weight 100,000 cut-off) and the particles were purified by affinity column chromatography using monoclonal antibody and by cesium chloride density-gradient centrifugation. In a typical experiment, HBsAg particles containing 1 mg of protein were obtained from 2 l of culture medium of huGK-14 cells or from 10 l of culture medium of PLC/PRF/5 cells. SDS-polyacrylamide gel electrophoresis analysis of the HBsAg particle preparations from both huGK-14 and PLC/PRF/5 cell showed that they contained only the polypeptides encoded by S-gene and pre-S2 gene, and no other proteins bands were detected (data not shown).

Lipid analysis

Total lipids of HBsAg particles corresponding to 5 mg of protein were extracted by the method of Bligh and Dyer (22). Cells (10^8) were harvested and washed three times with saline; total lipids were extracted as described above. The total lipid contents were determined gravimetrically. Dried lipid extracts were taken up to approximately 20 mg of lipids/ml in chloroform–methanol 2:1 (v/v) and stored under nitrogen at −20°C until used for further analysis. Phospholipids, diacylglycerol, triacylglycerol, cholesterol, and cholesteryl ester were separated by thin-layer chromatography (TLC) (DC-Fertigplatten Kieselgel 60, Merck) using n-hexane–diethyl ether–acetic acid 70:30:1 (v/v) as a developing solvent. After development, the plates were sprayed with a 0.002% primuline solution in acetone–water 5:1 (v/v), and lipid bands were visualized under UV light. The amounts of triacylglycerol and diacylglycerol were determined from the quantity of fatty acyl moieties. Fatty acids were analyzed as the methyl ester by gas–liquid chromatography using a glass column packed with 20% EGSS-X on chromosorb WAW. The methyl ester of 15:0 fatty acid was used as an internal standard. Cholesterol was quantified by gas–liquid chromatography using 3% SE-300 on chromosorb WAW (23). Cholestane was used as an internal standard. The amounts of cholesteryl ester were determined by the method of Heider and Boyett (24).

Subclass analysis of phospholipids

Individual phospholipids were separated by two-dimensional TLC with the first solvent system of chloroform–methanol–acetic acid 65:25:13 (v/v) and the second solvent system of chloroform–methanol–88% formic acid 65:25:10 (v/v) (25). They were extracted by the method of Bligh and Dyer and processed for phospholipid analysis as described below. Lipid phosphorus was estimated as described previously (26). 1-Alkenyl-2-acyl, 1-alkyl-2-acyl, and 1,2-diacyl GPC or GPE were separated as described (27). Briefly, phosphatidylcholine or phosphatidylethanolamine was hydrolyzed with phospholipase C (*B. cereus*)

and the resultant diradyl glycerol was extracted and subsequently acetylated. Three types of 1,2-diradyl-3-acetyl-glycerols were separated from each other on TLC plates developed in petroleum ether–diethyl ether–acetic acid 90:10:1 (v/v) and then with toluene. They were extracted from plates and the amounts of 1-alkenyl-2-acyl, 1-alkyl-2-acyl, and 1,2-diacyl compounds were estimated from the amount of fatty acids as described previously (28).

Assay of dihydroxyacetone-phosphate acyltransferase

DHAP-ATase assay was performed by the method of Schlossman and Bell (29), using ^{32}P-labeled dihydroxyacetone-phosphate and palmitoyl-CoA. [^{32}P]Dihydroxyacetone-phosphate was prepared enzymatically by the glycerol kinase (Boehringer Mannheim)-dependent phosphorylation of dihydroxyacetone according the method of Hajra (30). Cell suspensions were prepared as described by Zoeller and Raetz (31). Briefly, cells were harvested prior to confluency and after cells had been washed three times with PBS, they were resuspended in 5 volumes of 0.25 M sucrose, 20 mM Tris-HCl (pH 7.4), 0.5 mM dithiothreitol, 1.0 mM EDTA, and 0.02% sodium azide. The cell suspensions were briefly sonicated (10 sec) by the Branson Sonifier 185 and the resulting cell suspensions were used for the measurement of dihydroxy-acetone-phosphate acyltransferase activities. All assay mixtures consisted of 100 mM N-tris[hydroxymethyl]-methyl-2-aminoethane-sulfonic acid (pH 7.4), 100 μM palmitoyl-CoA, 1.5 mM [^{32}P]dihydroxyacetone-phosphate (2 μCi/μmol), 8 mM NaF, 5 mM $MgCl_2$, 50 mM KCl, 2 mg/ml of bovine serum albumin, and cell proteins in a total volume of 300 μl. All incubations were carried out at 37°C for 60 min. The activity of the enzyme, measured as the amount of product formed in 1 h, was linear with the amount of protein added at least up to concentration of 700 μg/ml. Effect of glycerol-3-phosphate or N-ethylmaleimide on the activity of dihydroxyacetone-phosphate acyltransferase was investigated in the same assay mixture as described above except that 5 mM of either glycerol-3-phosphate or N-ethylmaleimide was included.

TABLE 1. Lipid compositions of HBsAg particles and their host cells

	HBsAg Particles from		Host Cells	
Lipid	huGK-14	PLC/PRF/5	huGk-14	PLC/PRF/5
PL	90.6 ± 4.0	92.4 ± 2.5	42.7 ± 3.9	40.9 ± 1.2
DG	ND	ND	8.4 ± 0.4	4.0 ± 0.6
TG	4.4 ± 2.1	2.0 ± 0.2	40.6 ± 4.5	41.6 ± 2.0
CH	3.7 ± 1.8	4.1 ± 0.6	6.6 ± 3.2	9.7 ± 0.5
CHE	1.2 ± 0.4	1.5 ± 0.3	2.1 ± 1.1	3.8 ± 0.3

Total lipids from HBsAg particles corresponding to 5 mg of protein or 10^8 host cells were extracted by the method of Bligh and Dyer (22). Each class of lipids was separated and quantified as described in Methods. Data are presented as mole % of total amount of lipids and each value represents the mean ± SD of three different preparations; ND, not detected.

TABLE 2. Phospholipid compositions of HBsAg particles and their host cells

	HBsAg Particles from		Host Cells	
Phospholipid	huGK-14	PLC/PRF/5	huGk-14	PLC/PRF/5
PC	83.6 ± 2.4	85.7 ± 1.8	52.0 ± 0.6	50.2 ± 1.0
PE	7.2 ± 0.6	7.3 ± 0.7	29.7 ± 0.1	31.0 ± 0.3
PI	5.3 ± 2.5	3.9 ± 0.5	5.5 ± 0.2	5.3 ± 0.2
SM	2.9 ± 1.9	3.1 ± 1.1	6.7 ± 0.1	7.1 ± 0.2
PS	ND	ND	1.7 ± 0.1	2.5 ± 0.3
CL	ND	ND	4.6 ± 0.2	3.9 ± 0.1
L-PC	1.0 ± 0.9	ND	ND	ND

Data are presented as mole % of total amount of phospholipids and each value represents mean ± SD of three different preparations; ND not detected.

RESULTS

Comparison of lipid compositions between HBsAg particles and the host cell line

Purified HBsAg particles from both huGK-14 and PLC/PRF/5 cells showed a homogeneous population at an average diameter of 23 nm by electron micrographic observations and a density of 1.20 g/cm^3 by cesium chloride density gradient centrifugation (data not shown). The lipid to protein ratio for the average of three different preparations was 0.345 ± 0.031 and 0.349 ± 0.024 by weight for the HBsAg particle from huGK-14 cells and that from PLC/PRF/5 cells, respectively. **Table 1** shows the lipid compositions of the HBsAg particles and those of the host cell lines. More than 90% of the envelope lipids of HBsAg particles from both huGK-14 and PLC/PRF/5 cells were composed of phospholipid, whereas the host cells contained almost equal amounts of phospholipid and triacylglycerol. Phospholipid compositions of HBsAg particles and those of the host cell lines are shown in **Table 2**. Particles from both huGK-14 and PLC/PRF/5 cells showed an almost identical phospholipid composition; PC was the dominant phospholipid, accounting for more than 80%, followed by small amounts of PE, PI, and SM. Neither PS nor CL was detected in the preparations. In contrast, the host cells contained largely PC and PE, about 50% and 30%, respectively, along with small amounts of PI, SM, PS, and CL.

Subclass analysis of phospholipid

Table 3 shows subclass compositions of two major phospholipids, PC and PE, from the HBsAg particles and the host cell lines. In the HBsAg particles, more than 90% of PC and PE was composed of 1,2-diacyl glycerolphos-

TABLE 3. Subclass compositions of choline- and ethanolamine-containing glycerophospholipids of HBsAg particles and their host cells

Glycerophospholipid	HBsAg Particles from		Host Cells	
	huGK-14	PLC/PRF/5	huGk-14	PLC/PRF/5
GPC				
1-Alkenyl-2-acyl	5.1 ± 1.5[a]	2.3[b]	7.8 ± 1.0	35.6 ± 8.3
1-Alkyl-2-acyl	1.4 ± 0.4	6.8	56.3 ± 2.0	31.0 ± 2.9
1,2-Diacyl	93.6 ± 11.3	90.9	35.9 ± 1.2	33.4 ± 3.1
GPE				
1-Alkenyl-2-acyl	7.4 ± 3.8	7.1	33.9 ± 2.1	36.8 ± 3.9
1-Alkyl-2-acyl	5.7 ± 2.0	10.4	40.1 ± 3.8	44.3 ± 3.8
1,2-Diacyl	86.9 ± 9.1	82.5	26.0 ± 1.7	18.9 ± 2.0

[a]The results, expressed as percentage of total amount of PC or PE, are mean ± SD of three different preparations.

[b]The results were obtained from a single preparation. The values were calculated from the quantities of fatty acyl moieties.

phocholine (GPC) and 1,2-diacyl glycerophosphoethanolamine (GPE), respectively. In contrast, the host cells contained remarkably high amounts of ether-linked phospholipids; in huGK-14 cells 56.3% and 7.8% of PC were 1-alkyl-2-acyl GPC and 1-alkenyl-2-acyl GPC, respectively, whereas 40.1% and 33.9% of PE were 1-alkyl-2-acyl GPE and 1-alkenyl-2-acyl GPE, respectively. Similar high contents of ether-linked lipids were observed with PLC/PRF/5 cells (Table 3). The results clearly indicate that in both HBsAg particles, 1,2-diacyl GPC was preferentially incorporated into the HBsAg particles. The fatty acid compositions of 1,2-diacyl GPC and 1,2-diacyl GPE, both of which were obtained from the HBsAg particles from huGK-14 cells, resembled each other and were almost identical with those observed with the host cells (**Table 4**). A similar result was obtained with PLC/PRF/5 cells and HBsAg particles derived from the cells (data not shown). The major fatty acids of diacylglycerol, triacylglycerol, and cholesteryl ester of the HBsAg particles also resembled those of the host cells and the major components were palmitic (16:0), palmitoleic (16:1), stearic (18:0), and oleic (18:1) acids (data not shown).

Lipid compositions of human hepatoma cell lines

Both huGK-14 and PLC/PRF/5 cells were found to contain surprisingly high levels of ether-linked phospholipids compared to normal hepatocytes which are known to contain almost exclusively glycerophospholipids of 1,2-diacyl type (15). Comparison of the phospholipid compositions of different hepatoma cell lines was next done to determine a possible relationship between HBV infection and altered lipid metabolism. It is known that huGK-14 (12) and PLC/PRF/5 (13) cells are persistently infected by HBV and contain integrated HBV DNA in their chromosomes; other human hepatoma cell lines, such as HuH-7 (19), huL-1(21), and Hep-G2 (20, 32), were established from hepatocellular carcinomas which were not associated with HBV infection. No significant difference in phospholipid compositions was observed among the cell lines examined, in which the major phospholipids were PC and PE (data not shown). Analysis of the subclass compositions of PC and PE revealed that the two hepatoma cell lines which produce HBsAg particles (huGK-14 and PLC/PRF/5) contained higher amounts of 1-alkyl or

TABLE 4. Major fatty acids of choline- and ethanolamine-containing diacylglycerophospholipids of HBsAg particles and huGK-14 cells

Fatty Acids	PC		PE	
	HBsAg Particle	huGK-14	HBsAg Particle	huGK-14
14:0	1.4 ± 0.7	1.3 ± 0.3	1.7 ± 0.8	0.5 ± 0.1
16:0	28.1 ± 4.8	31.5 ± 8.4	13.3 ± 4.6	18.1 ± 5.8
16:1	15.7 ± 1.9	21.3 ± 8.2	8.5 ± 3.2	11.8 ± 2.6
18:0	4.4 ± 1.3	4.4 ± 0.9	19.0 ± 4.5	20.9 ± 0.7
18:1	50:0 ± 8.9	41.1 ± 4.9	54.0 ± 11.9	44.9 ± 12.8
20:4	0.4 ± 0.3	0.4 ± 0.1	3.7 ± 0.9	3.8 ± 1.0

The results, expressed a percentage of total fatty acids, are the mean ± SD of three different preparations.

TABLE 5. Subclass compositions of choline- and ethanolamine-containing glycerophospholipids of human hepatoma cell lines

Glycerophospholipid	HuH-7	huL-1	Hep-G2
GPC			
1-Alkenyl-2-acyl	1.7 ± 0.7	1.2 ± 1.0	ND
1-Alkyl-2-acyl	6.2 ± 1.5	4.0 ± 2.7	0.4 ± 0.1
1,2-Diacyl	92.1 ± 7.3	94.8 ± 8.3	99.6 ± 0.5
GPE			
1-Alkenyl-2-acyl	8.7 ± 1.1	4.1 ± 0.9	3.3 ± 1.2
1-Alkyl-2-acyl	4.1 ± 0.8	7.6 ± 1.2	1.4 ± 0.7
1,2-Diacyl	87.2 ± 9.4	88.3 ± 10.3	95.3 ± 2.3

The results, expressed as percentage of total amount of PC or PE, are the mean ± SD of three different preparations. The values were calculated from the quantities of fatty acyl moieties; ND, not detected.

1-alkenyl type glycerophospholipids (Table 3), whereas the other hepatoma cell lines (HuH-7, Hep-G2, and huL-1) or normal human liver cells (15) contained mostly 1,2-diacyl type glycerophospholipids (**Table 5**). The two cell lines (huGK-14 and PLC/PRF/5) had been grown in either serum-free media (huGK-14) or media with low FCS concentration (PLC/PRF/5), while other cell lines had been grown in media containing 10% FCS. Analysis of the phospholipids of huGK-14 and PLC/PRF/5 cells grown in media containing 10% FCS showed no significant change of the subclass compositions of phospholipids, suggesting that the difference observed with these cell lines was not due to the different serum concentrations of the media.

Dihydroxyacetone-phosphate acyltransferase activities in human hepatoma cell lines

Since acylation of dihydroxyacetone-phosphate is generally accepted as an obligatory step in ether lipid synthesis (33–36), we next examined the activities of dihydroxyacetone-phosphate acyltransferase in the human hepatoma cell lines. Specific activities are summarized in **Table 6**, showing that the two human hepatoma cell lines (huGK-14 and PLC/PRF/5) with higher amounts of ether-linked phospholipids had three- to fourfold higher dihydroxyacetone-phosphate acyltransferase activities than the other two cell lines (HuH-1 and huL-1). In liver, dihydroxyacetone-phosphate acyltransferase appears to be located in peroxisomes and microsomes, and the enzyme activities measured at pH 7.4 should reflect both the peroxisomal and microsomal enzyme activties (36). A slight increase of the dihydroxyacetone-phosphate acyltransferase activities was observed when an assay was performed at pH 5.5, which was optimal for the peroxisomal enzyme activity (36); specific activities were 1412 ± 74.7 (pmol/min/mg protein) for huGK-14 cells and 1051 ± 109 (pmol/min/mg protein) for PLC/PRF/5 cells. Since microsomal dihydroxyacetone-phosphate acyltransferase was almost completely inhibited by glycerol-3-phosphate and N-ethylmaleimide (36), the effects of both agents on the enzyme activity were investigated. Specific activities of dihydroxyacetone-phosphate acyltransferase measured in the presence of 5 mM glycerol-3-phosphate or 5 mM N-ethylmaleimide at pH 7.4 are shown in Table 6. Neither glycerol-3-phosphate nor N-ethylmaleimide showed any significant effect on the enzyme activities,

TABLE 6. Dihydroxyacetone-phosphate acyltransferase activities in various human hepatoma cell lines

Addition	Activity in			
	huGK-14	PLC/PRF/5	HuH-7	huL-1
	pmol/min/mg protein			
	950.5 ± 18.7	862.2 ± 16.2	372.3 ± 3.9	207.1 ± 54.1
G-3-P, 5 mM	820.9 ± 18.6	654.8 ± 24.3	136.4 ± 14.8	114.3 ± 18.7
NEM, 5 mM	1123 ± 227	924.2 ± 30.4	367.7 ± 28.4	230.2 ± 69.3

G-3-P, glycerol-3-phosphate; NEM, N-ethylmaleimide. Whole cell lysates were assayed at 37°C. All assay mixtures consisted of 100 μM N-tris[hydroxymethyl] methyl-2-aminoethanesulfonic acid (pH 7.4), 100 mM palmitoyl-CoA, 1.5 mM [^{32}P]dihydroxyacetone-phosphate (2 μCi/μmol), 8 mM NaF, 5 mM $MgCl_2$, 50 mM KCl, 2 μM KCN, 2 mg/ml of bovine serum albumin, and cell proteins in a total volume of 300 μl. Incubations were carried out at 37°C for 60 min. Effect of G-3-P or NEM was investigated in the same assay mixture except that 5 mM of either G-3-P or NEM was included. All values represent the mean ± SD of two separate preparations.

suggesting that the peroxisomal enzyme appears to be responsible for most of the elevated levels of dihydroxyacetone-phosphate acyltransferase in both huGK-14 and PLC/PRF/5 cells.

DISCUSSION

Phospholipids assembled into the HBsAg particles

Although the HBsAg particles produced by the human hepatoma cell lines resembled the HBsAg particles from patient plasma in morphology, physical characteristics, and protein composition (12, 37), certain differences in lipid composition were observed between cultured cell-derived and patient plasma-derived HBsAg particles. The HBsAg particles from patient plasma were reported to contain relatively high concentrations of cholesterol and cholesteryl ester, accounting for 16 mol% and 14 mol% of the total lipids, respectively, and there was some resemblance to human high density lipoprotein in lipid composition (10). The difference in cholesterol and cholesteryl ester contents may reflect the difference between nascent (cultured cell-derived) and circulating (patient serum-derived) HBsAg particles. The exchange and transfer, as well as the remodeling of the HBsAg particles during the circulation, may cause such an alteration.

By comparing the lipids of various purified viruses with those of the isolated host membranes at which they had been assembled, we are confident that the phospholipid compositions of the viral envelope were essentially the same as those of the host cell membranes (6, 9). Electron microscopic analysis of the liver explants from infected patients and that of cultured cell lines that had been stably transformed with the S gene of HBV have indicated that the assembly and packing of HBsAg particles occur in the endoplasmic reticulum (ER) and the mature HBsAg particles are secreted via the vesicular pathway (38, 39). Although the site of the assembly of the HBsAg particles in huGK-14 cells is still under investigation and we could not directly compare the lipid compositions between the particles and the membranes where they assembled, the present results indicate that, among various classes of lipids present in the host cell membranes, 1,2-diacyl GPC was preferentially incorporated into the membrane of the HBsAg particles. The lipid compositions of HBsAg particles showed a clear difference from those of ER membranes of various tissues (40) and other viruses that have been reported to assemble intracellularly (6, 41). Since the cultured cell lines that had been stably transformed with the S gene alone can assemble and secrete HBsAg particles in the absence of other viral proteins, presence of the major viral surface antigen (S) proteins encoded by S gene may direct the assembly and the secretion of the particles (38, 39). In our preliminary experiments, the preferential usage of 1,2-diacyl GPC was also observed with other HBsAg particles produced by mouse fibroblast cells transfected with cloned hepatitis B virus DNA (Imai, H., M. Umeda, H. Utsumi, T. Yomeyama, T. Miyamura, O.Satoh, H. Tunoo, and K. Inoue, manuscript in preparation). This suggests that the lipid composition of HBsAg particles was not dependent on the nature of the host cell line. The particles may bud from the restricted area in the ER where 1,2-diacyl GPC is segregated. Alternatively, HBsAg S proteins themselves might be responsible for the preferential incorporation of 1,2-diacyl GPC into the particles.

Aberrant lipid compositions of human hepatoma cells persistently infected by HBV

Researchers have suggested an association between tumors and altered lipid metabolism (14, 16, 18). Particularly, elevated levels of ether-linked neutral lipids have been strongly associated with malignancy and tumorigenicity (17). Tumorous brain and hepatomas often showed elevated levels of ether-linked glycerophospholipids compared to normal tissues, whereas lung carcinoma did not show any change (42–44). The mammalian hepatoma cell lines had higher levels of ether-linked glycerophospholipids; the contents were 1.8% and 5.6% of total glycerophospholipids in Morris hepatoma and in Yoshida ascites hepatomas, respectively (43). In the present study, we found extremely elevated levels of ether-linked glycerophospholipids (more than 60% of total glycerophospholipids) in two hepatoma cell lines, huGK-14 cells and PLC/PRF/5 cells. In contrast, only a slight increase was observed with other human hepatoma cell lines, HuH-7, Hep-G2, and huL-1 (Table 3 and Table 5). Both huGK-14 (12) and PLC/PRF/5 (32) are the human hepatocarcinoma cell lines that have been persistently infected by HBV, while other hepatoma cell lines such as HuH-7 (19), Hep-G2 (20, 32), and huL-1 (21) were established from human hepatocellular carcinomas that were not associated with HBV infection. The results, so far obtained, clearly indicate that HBV-induced hepatocellular carcinogenesis was associated with massive changes in ether lipid metabolism above non-HBV-related hepatocellular carcinogenesis. The mechanism of oncogenesis by HBV is not known but integration of HBV DNA into the host genome is thought to be an important factor (45). It is possible that the transfection by HBV or integration of the viral DNA into the host chromosomes may alter the reactivities of host cell enzymes that are responsible for the synthesis of the ether-linked glycerophospholipids (33–36). Elevated activity of dihydroxyacetone-phosphate acyltransferase, which is known to be an obligatory enzyme in ether-lipid biosynthesis (34, 36), was observed with both huGK-14 and PLC/PRF/5 cells. Other key enzymes (14), such as the alkyldihydroxyacetone-phosphate synthase, or the enzymes governing fatty alcohol levels or the enzymes involved in degradation of ether-lipids could also be

affected. Although the functional role of ether-linked phospholipids in cell membranes remains obscure, the present study may provide some clues about the significance of HBV infection and the aberrant metabolism of membrane lipids, and conceivably, about the mechanism of HBV-induced hepatocellular carcinogenesis.

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