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MODIFICATION OF THE FATTY ACID COMPOSITION OF PHOSPHOLIPID IN MEASLES VIRUS-PERSISTENTLY INFECTED CELLS

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SUMMARY

Thé composition of phospholipid was studied in BGM cells uninfected, persistently infected or lytically infected with measles virus, strain Hallé. In persistently infected cells, phosphatidylcholine palmitic acid content, and phosphatidylethanolamine palmitic acid and arachidonic acid contents were significantly increased. Lytically infected cells had a similar phospholipid fatty acid composition to the uninfected. Phosphatide composition showed minor modifications, but the content of total choline-derivative phospholipids was unchanged in either type of infection.

Phospholipids, by virtue of their amphipathic character and assymetric distribution, are responsible for the structural integrity of the cell membrane. Modifications of the phosphatide composition of phospholipid in cells may arise as a consequence of virus infection (1,2). Virus infection also affects fatty acid composition (2,3). Recently, the unsaturated fatty acid content of the host cell has been correlated to virulence (4).

In this communication we have analysed the phosphatide and fatty acid compositions of phospholipid in uninfected and measles-virus infected monkey kidney cells, BGM and BGM/Hallé. Two different types of infection, (1) a lytic productive infection, and (2) a persistent infection (4,5), were studied.

MATERIALS AND METHODS

Cells : African Green Monkey cells (BGM) uninfected, or persistently infected with measles virus strain Hallé were grown in monolayers as

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previously described (4). A lytic infection was established by inoculation with Hallé at a multiplicity of 0.1 p.f.u./cell. Immediately prior to analysis the medium was supplemented with a specified batch of foetal calf serum (GIBCO) at a concentration of 2 %. This was changed daily.

Liquid extraction : The cell monolayers were washed twice and scraped down in 0.14M NaCl, then centrifuged at 500 g for 10 min. Lipid was extracted by the method of Folch (6) modified as follows : $5X10^7$ cells were suspended in 30 ml chloroform/methanol (2:1 v/v) and left to stand for 45 min in the dark. The organosoluble material was washed successively with (1) 6 ml 0.2 % KCl (2) 6 ml 0.2 % KCl, 10 ml methanol (3) 6 ml distilled water, 10 ml methanol. Biphasic separation was carried out by centrifugation (1000 g for 5 min). The organic phase was evaporated to dryness under vacuum (60°C) in a preweighed round-bottomed flask. The total lipid extract was weighed, resuspended in 2 ml benzene/methanol (2:1 v/v) and stored at - 70° C.

Thin layer chromatography : The lipid extracts were resolved into their consistent phosphatides by thin-layer chromatography on ultra-pure silica gel glass-backed plates (Merck). The solvent systems were as described previously (3,7). Dittmer's reagent (8) was used to locate phosphatides to be estimated for lipid phosphorous. Primulin (8) was used to locate co-migrating standards when phospholipids were prepared for fatty acid analysis.

Estimation of lipid phosphorous : Either the total lipid extract, or separated phosphatides were analysed for organic phosphate content by the method of Bartlett (10) modified to increase sensitivity (11).

Gas-liquid chromatography : Fatty acids were prepared for GLC by transmethylation in screw-capped tubes (12,13). The fatty acid methyl esters were eluted by shaking with 5 ml hexane. Silica gel was removed from phosphatide samples by the addition of 4 ml water and centrifugation (1000 g, 5 min). The upper (organic) phase was removed, evaporated to dryness under nitrogen at room temperature, and resuspended in 0.1 ml hexane. An aliquot of 5 ul was injected into a 0.22X3 m stainless steel column packed with 10 % DEGS on 80/100 mesh WAW chromosorb, at 190°C. Fatty acid methyl esters were identified by comparison with standards and their composition calculated by the planimetric method.

RESULTS

No change was observed in the percentage weight of phospholipid/total lipid in lytically or persistently infected cells compared to the uninfected (results not shown). The percentage composition by weight of the phosphatides is shown in Table 1. Persistently infected cells show a marked increase in lysophosphatidylcholine content, and a decrease in phosphatidylethanolamine content compared to uninfected cells, but the PC/SM molar ratio (2.49 vs 2.72) and the content of total choline-derivative phospholipids (61.5 vs 58.4) are unchanged. Lytically infected cells are similarly decreased in phosphatidylethano-

Table 1

PHOSPHATIDE COMPOSITION OF PHOSPHOLIPID IN UNINFECTED AND MEASLES-INFECTED MONKEY KIDNEY CELLS (BGM/Hallé)

Phospholipid	Uninfected	Lytically infected	Persistently infected
Lysophosphatidyl -choline	1.8	0.5	2.9
Sphingomyelin	15.2	10.4	16.8
Phosphatidyl -choline	41.4	53.0	41.8
Phosphatidyl -ethanolamine	29.5	25.2	25.6
Phosphatidyl -serine	3.8	1.6	2.8
Phosphatidyl -inositol	4.2	2.7	4.8
Diphosphatidyl -glycerol	4.2	6.7	4.8

Uninfected and lytically infected cells were harvested at confluence. Lytically infected cells were harvested when showing a cytopathic effect of ++. The cells were scraped down in 0.14M NaCl, centrifuged, and extracted for lipid as described in Materials and Methods. The results are expressed as a percentage of the total lipid phosphate.

lamine content compared to uninfected cells, and contain less lysophosphatidylcholine. The PC/SM molar ratio is increased in these cells (5.09), but the total choline-phospholipid content is again maintained (63.9).

The fatty acid composition of total phospholipid is shown in Table 2. Persistently infected cells are markedly increased in content of palmitic acid (C16:0) and decreased in content of oleic acid (C18:1) compared to uninfected cells. No change was found in the fatty acid composition of the neutral lipid fraction (results not shown). Lytically infected cells have a phospholipid fatty acid composition resembling that of uninfected cells, apart from a reduction in arachidonic acid (C20:4) content.

Table 2

FATTY ACID COMPOSITION OF PHOSPHOLIPID IN UNINFECTED AND MEASLES-INFECTED MONKEY KIDNEY CELLS (BGM/Hallé)

Fatty acid	Uninfected	Lytically infected	Persistently infected
16.0	24.1	23.3	30.9
16.1	4.1	5.1	6.1
18.0	17.1	17.3	16.2
18.1	40.8	42.6	27.8
18.2	1.4	2.9	6.0
18.3	Tr	Tr	Tr
20.1	Tr	Tr	Tr
20.3	2.0	1.9	1.2
22.1	0.2	0.8	1.1
20.4	8.6	4.7	9.9
20.5	1.6	1.4	0.7
Saturated fatty acids	41.2	40.6	47.1

All cells were cultured in medium containing 2 % foetal calf serum of defined fatty acid composition. Harvesting and lipid extraction was carried out as described for Table 1. Phospholipid was separated from the neutral lipid fraction by one-dimensional thin layer chromatography in diethyl ether. Transmethylation and gas-liquid chromatography were carried out as described in Materials and Methods. The results are expressed as a percentage of the total fatty acid (standard error 5 %). Polyunsaturated C:22 fatty acids, and peaks representing less than 0.5 % of the total, were not included.

In order to examine fatty acid distribution among the major constituent phosphatides, we resolved PC and PE from the total phospholipid fraction and analysed their fatty acid composition. Persistently infected cells contain PC which is also increased in palmitic acid and decreased in oleic acid content compared to lytically infected and uninfected cells (Table 3). This change of composition is paralleled by PE in persistently infected cells, whose arachidonic acid content is also

Table 3

FATTY ACID COMPOSITION OF PHOSPHATIDYLCHOLINE IN UNINFECTED AND MEASLES-INFECTED MONKEY KIDNEY CELLS (BGM/Hallé)

Fatty acid	Uninfected	Lytically infected	Persistently infected
16.0	32.6	27.5	42.6
16.1	5.0	5.6	8.0
18.0	13.7	15.3	11.7
18.1	40.1	42.7	26.3
18.2	1.9	1.1	3.7
18.3	Tr	Tr	Tr
20.1	Tr	Tr	Tr
20.3	1.7	1.9	1.9
22.1	0.4	0.2	0.7
20.4	3.7	4.5	4.9
20.5	0.9	1.2	0.2
Saturated fatty acids	46.3	42.8	54.3

The conditions for analysis were the same as those described in Table 2.

elevated (Table 4). The increase in palmitic acid content in persistently infected cells appears to be of the same order of magnitude for total phospholipid, PC and PE.

DISCUSSION

Persistent morbillivirus infections in vitro are often characterized by an alteration in the expression of viral proteins, with decreased synthesis of the envelope polypeptides. However, our previous experiments comparing persistent and lytic BGM/Hallé infections have found no change in the quantities of the HA and F1 antigens synthesised and exposed at the cell surface (5,14). Here, we have shown that the contents of palmitoyl-phosphatidylcholine, palmitoyl-phosphatidylethanolamine and arachidonyl-phosphatidylethanolamine increase in persistent-

Table 4

FATTY ACID COMPOSITION OF PHOSPHATIDYLETHANOLAMINE IN UNINFECTED AND MEASLES-INFECTED MONKEY KIDNEY CELLS (BGM/Hallé)

Fatty acid	Uninfected	Lytically infected	Persistently infected
16.0	10.7	11.6	13.4
16.1	6.1	4.5	5.9
18.0	22.7	23.4	23.1
18.1	33.7	45.2	23.4
18.2	2.2	3.2	1.9
18.3	0.4	1.2	0.6
20.1	1.5	0.5	0.9
20.3	Tr	Tr	Tr
22.1	Tr	Tr	Tr
20.4	19.5	9.1	27.6
20.5	3.1	1.4	2.1
Saturated fatty acids	33.4	35.0	36.5

The conditions for analysis were the same as those described in Table 2.

ly infected cells. These compositional changes may arise as a result of selection pressure for a subset of cells tolerant of persistent infection, or as a modification of cellular metabolism. Similar increases in palmitic and arachidonic acid contents of polar lipids were noted in rabies virus infection of Nil 2-monolayer cultivated cells, and in released virus (15). As persistently infected cells continue to release infectious virus, we are comparing its phospholipid and fatty acid compositions with those of virus from lytically infected BGM/Hallé cells, in order to see whether the differences observed in the host are preserved.

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