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PHOSPHOLIPID MODIFICATION RETARDS INTRACELLULAR TRANSPORT AND SECRETION OF IMMUNOGLOBULIN G1 BY MOUSE MOPC-31C PLASMACYTOMA CELLS

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The intracellular transport and secretion of immunoglobulin G1(IgG1) by mouse MOPC-31C plasmacytoma cells were analyzed from the viewpoint of the roles of phospholipids. The membrane phospholipids were modified by culturing cells in a medium supplemented with choline analogues, *N*,*N*′-dimethylethanolamine or *N*-monomethylethanolamine, and accordingly the membranes were enriched in phosphatidyl-*N*,*N*′-dimethylethanolamine or phosphatidyl-*N*-monomethylethanolamine (Maeda, M., Tanaka, Y. and Akamatsu, Y. (1980) Biochem. Biophys. Res. Commun. 96, 876–881). The modified cells were pulse-labeled with L-[^{35}S]methionine and the secretion of labeled IgG1 was chased. Half of the IgG1 was exported to the extracellular medium 1–1.5 h and 2–3 h after synthesis by choline- and dimethylethanolamine-supplemented cells, respectively. However, most of the newly synthesized IgG1 was not secreted by monomethylethanolamine-supplemented cells, even after 5 h; it remained within the cells. The sensitivity of intracellular IgG1 to endoglycosidase H was examined for probing the movement of IgG1 from the rough endoplasmic reticulum to the Golgi complex. Half of the newly synthesized IgG1 acquired resistance to endoglycosidase H after 30–45 min, 1–1.5 h and 2–3 h in choline-, dimethylethanolamine- and monomethylethanolamine-supplemented cells, respectively. Thus, the transport of IgG1 was markedly retarded by the modification with choline analogues, dimethylethanolamine or monomethylethanolamine, at least in the following two processes, from the rough endoplasmic reticulum to the Golgi complex and from the Golgi to the outside of cells. Modification with monomethylethanolamine was more effective than that with dimethylethanolamine in slowing down the transport of IgG1 and appeared to cause accumulation of IgG1 within the cells. A morphological study was also carried out for the three kinds of cell. The roles of phospholipids in the processes of membrane flow are discussed.

Introduction

Secretion of immunoglobulin by plasma cells has been well investigated as a typical example of protein transport [1–7]. Immunoglobulin is synthesized on ribosomes attached to the rough endoplasmic reticulum, segregated into the cisternal space of the rough endoplasmic reticulum, transported to the Golgi complex, and finally secreted to the outside of cells probably by exocytosis on the plasma membrane. In all of these processes, membrane systems are essentially involved. Most studies on these processes have been focused on the nature of the transported proteins themselves, especially on the oligosaccharide moiety [4,5,7]. Recently, some membrane-associated proteins have been characterized and found to be essential for the translocation of nascent polypeptides across the rough microsomal mem-

Abbreviations: DME, *N*,*N*′-dimethylethanolamine; ME, *N*-monomethylethanolamine; SDS, sodium dodecyl sulfate.

branes of dog pancreas [8,9]. Genetics have also proved a powerful tool for studying the machinery of protein transport [10]. However, only a few reports [11–13] have been presented on the roles of the alternative constituents of membranes, lipids.

Phospholipid bilayers not only surround membrane proteins but also control their environments by regulating the fluidity of membranes. Furthermore, positive roles of phospholipid metabolism have been postulated in some functions of biomembranes such as responses to hormones [14,15]. Cultured cell lines are suitable systems for the approach from lipid biochemistry because many variables such as phospholipid composition, fatty acid composition and cholesterol content of the membranes can be modified by altering the culture conditions [16–19]. Cellular functions associated with membranes are expected to be modulated by these modifications. Actually, Schroeder [20] has recently reported that the endocytotic function of mouse LM cells was inhibited by alteration of their phospholipid composition.

In the present study, we examined the intracellular transport and secretion of immunoglobulin G1 (IgG1) by mouse MOPC-31C plasmacytoma cells from the viewpoint of the roles of phospholipids. This cell line can be maintained in a defined medium so that the phospholipid composition of membranes is readily modified by culturing cells in a medium supplemented with choline analogues, *N,N'*-dimethylethanolamine (DME) or *N*-monomethylethanolamine (ME) [21]. The transport of IgG1 in the cells treated with these choline analogues was revealed to be markedly retarded as compared with the control cells, at least in the following two processes, from the rough endoplasmic reticulum to the Golgi complex and from the Golgi to the outside of cells.

Materials and Methods

Cells and chemicals. Mouse MOPC-31C plasmacytoma cells [22] were obtained from Flow Laboratories (Rockville, MD) and maintained at 37°C as a suspension in Leibovitz's L-15 medium supplemented with 10% fetal calf serum (both from Flow Laboratories). L-[^{35}S]Methionine was from Amersham International and $H_3{}^{32}PO_4$ was from the Commissariat à l'Energie Atomique (Gif-sur-Yvette, France). Choline, DME and ME were purchased from Eastman. Mouse IgG and the IgG fraction of rabbit antiserum to mouse IgG were from Miles. Endoglycosidase H (endo-β-*N*-acetylglucosaminidase H) was obtained from Seikagaku Kogyo (Tokyo, Japan).

Modification of phospholipid with choline analogues. The phospholipid of MOPC-31C cells was modified by culturing cells in choline-depleted Higuchi medium [23] supplemented with 10% dialyzed fetal calf serum and 40 μg/ml choline, DME or ME for 3 days [21]. The phospholipid compositions of modified cells were determined as follows. Cells ($3.7 \cdot 10^5$/ml) were cultured with choline or one of its analogues as above except that the medium contained 2 μCi/ml [^{32}P]phosphate. After 3 days, cells were harvested, washed with phosphate-buffered saline and subjected to extraction of lipids according to Bligh and Dyer [24]. Extracted lipids were separated by two-dimensional thin-layer chromatography with chloroform/methanol/water (65:25:4, v/v) as solvent in the first dimension, and butanol/acetic acid/water (6:2:2, v/v) in the second [19]. Each spot was scraped off and the radioactivity was measured.

Labeling of cells. Pulse-chase experiments on MOPC-31C cells with modified phospholipid compositions were carried out as follows. The modified cells ($2 \cdot 10^6$/ml) were pulse-labeled with 50 μCi/ml [^{35}S]methionine (over 900 Ci/mmol) at 37°C for 10 min in Higuchi medium (−choline and −methionine) supplemented with 10% dialyzed fetal calf serum and 40 μg/ml choline or one of its analogues. Then 0.5 mM nonradioactive methionine was added and the incubation was continued. Aliquots (0.2 ml) of cell suspensions were withdrawn at appropriate intervals, chilled on ice and centrifuged at $2000 \times g$ for 5 min. The supernatant fraction was used for the analysis of secreted IgG1. The precipitated cells were washed with phosphate-buffered saline containing 10 mM methionine and lysed by treatment with 0.2 ml 1% Triton X-100 and 0.5 mM phenylmethylsulfonyl fluoride (Sigma) in phosphate-buffered saline for 30 min at 0°C. The lystate was clarified by centrifugation at $2000 \times g$ at 0°C for 30 min and subjected to immunoprecipitation.

Immunoprecipitation. For the analysis of intracellular IgG1, IgG1 in lysates was immunoprecipitated with rabbit antibody to mouse IgG. The IgG fraction (180 μg) of rabbit antiserum to mouse IgG was added to 10–20 μl lysates together with 2 μg mouse IgG as carrier. The reaction mixtures were incubated at 37°C for 1 h and then at 4°C overnight. Phosphate-buffered saline (1 ml) containing 10 mM methionine was added, and the immunoprecipitates were spun down at 2000 × *g* at 0°C for 30 min and dissolved in electrophoresis buffer. The second precipitation with formalin-fixed *Staphylococcus aureus* cells [25] or with Protein A-Sepharose CL-4B [26] was found to improve neither recovery of the antigen nor reproducibility of data in this case, and was therefore omitted from the procedure. Extracellular IgG1 was analyzed without immunoprecipitation, because only IgG1 was detected in the culture medium as a labeled protein in every experiment.

Electrophoretic analysis of IgG1. For determinating the IgG1 amount, SDS-polyacrylamide gel electrophoresis was carried out under non-reducing conditions so that IgG1 was analyzed as the whole H_2L_2 complex. Sample solutions in final concentrations of 50 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol and 0.001% Bromophenol blue were heated at 90°C for 5 min, alkylated with 20 mM iodoacetamide at room temperature in the dark for 30 min and applied on SDS-polyacrylamide 7.5–15% gradient gels using Laemmli's buffer system [27]. Electrophoresed gels were processed for fluorography as described [28] and dried. Fluorograms were obtained on Fuji Rx Medical X-ray films preexposed under a condition in which band intensity was directly proportional to radioactivity [29]. The developed films were scanned with a Joyce-Loebl densitometer equipped with a Hewlett-Packard Model 3385A integrator, and the relative peak area was used as a measure of the radioactivity of each band.

Treatment with endoglycosidase H. The sensitivity of oligosaccharide chains of IgG1 to endoglycosidase H was analyzed as follows. Samples dissolved in 20 μl of electrophoresis buffer were reduced by the addition of 20 mM dithiothreitol, heated and alkylated with 50 mM iodoacetamide as described above. Then 0.5 ml 10% trichloroacetic acid was added, and the precipitates were washed with ice-cold acetone and dissolved in 50 μl 0.3 M sodium citrate (pH 5.5)/0.1% SDS. Each solution was divided into two: one portion received 0.33 μg of endoglycosidase H, and both were incubated at 37°C for 14–20 h. Proteins were again precipitated with trichloroacetic acid, washed with cold acetone and redissolved in electrophoresis buffer to be analyzed on 9–15% gradient gels.

Electron microscopy. MOPC-31C cells whose phospholipid composition was modified with choline analogues were fixed at 0°C with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.15 M sodium cacodylate buffer (pH 7.5) for 30 min and then with 1% OsO_4 in the same buffer for 30 min. After dehydration with ethanol and propyrene oxide treatment, the cells were embedded in Epon 812 resin. The ultrathin sections (400–600 Å) were stained with 2% uranyl acetate for 30 min and with 0.2% lead citrate for 5 min and then examined under a Hitachi H-500 electron microscope.

Results

Modification of phospholipid composition with choline analogues

The phospholipid composition of MOPC-31C cells was easily modified by culturing cells in a medium containing choline analogues in place of choline. Under the conditions reported previously [21], cells grew almost normally for 3 days in the presence of 40 μg/ml DME or ME, and the corresponding phospholipid, phosphatidyl-*N*,*N'*-dimethylethanolamine or phosphatidyl-*N*-monomethylethanolamine, increased markedly in their membranes. In the present study, phospholipid compositions were altered more extensively because the culture medium of the previous study [21], containing 20% dialyzed calf serum, was replaced by that containing 10% dialyzed fetal calf serum, which could supply smaller amounts of phospholipids to cells than the former. As shown in Table I, choline-, DME- and ME-supplemented cells incorporated these bases into their membrane phospholipids and each of the corresponding phospholipids became the major component, occupying up to 60–70%. The composition of choline-supplemented cells was essentially the same as that of cells maintained in the usual medium. In

TABLE I

PHOSPHOLIPID COMPOSITION OF MOPC-31C CELLS CULTURED WITH CHOLINE OR ITS ANALOGUES

Cells were cultured in the presence of 40 μg/ml choline, *N,N'*-dimethylethanolamine (DME) or *N*-monomethylethanolamine (ME) for 3 days and the phospholipid compositions were analyzed as described under Materials and Methods. The percentage values represented were calculated from the ^{32}P-radioactivities of individual phospholipids. Abbreviations: PC, phosphatidylcholine; PDE, phosphatidyl-*N,N'*-dimethylethanolamine; PME; phosphatidyl-*N*-monomethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

Phospholipids	Supplement (%)		
	Choline	DME	ME
PC	60.9	3.9	16.3
PDE	0.2	67.6	1.6
PME	0.2	0.2	64.8
PE	19.2	9.9	2.7
PG+CL	10.3	8.3	4.4
PI+PS	6.3	7.8	4.3
SM	1.5	0.9	0.5
Others	1.4	1.4	5.4

spite of such extensive modification, the growth of DME-supplemented cells was almost the same as that of the control cells, whereas the growth rate of ME-supplemented cells was appreciably decreased. Nevertheless, this modification could provide an extreme condition for investigating the roles of phospholipids in the intracellular transport and secretion of IgG1 as will be described below.

Secretion of IgG1

The time courses of IgG1 secretion were determined for choline-, DME- and ME-supplemented cells. The three kinds of cell were pulse-labeled with [^{35}S]methionine for 10 min and then chased. The amount of IgG1 secreted into the medium and that remaining intracellularly during the chase period are shown in Fig. 1. In the case of the control choline-supplemented cells (Fig. 1A), the amount of intracellular IgG1 (open circles) reached maximum at about 30 min, indicating that the assembly of newly synthesized H and L chains was completed around this time, and then the

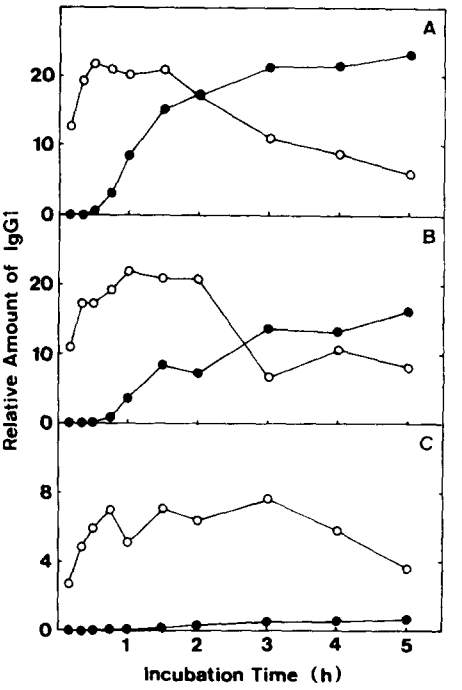

Fig. 1. Time courses of IgG1 secretion by MOPC-31C cells cultured with choline (A), DME (B) or ME (C). Cells were cultured for 3 days with 40 μg/ml choline or one of its analogues and then pulse-labeled with L-[^{35}S]methionine in Higuchi medium (−choline and −methionine) supplemented with 10% dialyzed fetal calf serum and 40 μg/ml choline or one of its analogues. After 10 min, nonradioactive methionine was added and the incubation was continued. Aliquots were withdrawn at the times indicated and subjected to analysis of extracellular (●) and intracellular (○) IgG1. Electrophoretic analysis of IgG1 was carried out under non-reducing conditions as described under Materials and Methods. The relative amount of IgG1 (arbitrary unit) was calculated from the band intensity measured by scanning of fluorograms. The incubation time is represented from the beginning of pulse-labeling.

intracellular IgG1 decreased gradually. On the other hand, IgG1 was detected in the extracellular medium after 30 min (closed circles) and half of the synthesized IgG1 molecules were found to be secreted at 1–1.5 h. Fig. 1B shows the time course with DME-supplemented cells, in which the secretion of IgG1 was significantly slowed down as compared with the control cells. 2–3 h was needed for half of the IgG1 molecules to be secreted extracellularly. The secretion of IgG1 was most markedly retarded in the case of ME-supplemented cells. As shown in Fig. 1C, less than 10% of labeled IgG1 molecules were exported into the medium, even after 5 h; most of them still re-

mained within the cells. These observations indicate that the modification of phospholipid composition with choline analogues had an inhibitory effect on IgG1 secretion by MOPC-31C cells. Such an effect was shown to be most striking for ME-supplemented cells. Another experiment in which the secretion of IgG1 was chased for a longer period revealed that half of the synthesized IgG1 was found in the extracellular medium after as long as 15 h (data not shown). This suggests that ME-supplemented cells did not lose the ability to export IgG1 though the intracellular transport and/or the exocytotic process of IgG1 were greatly slowed down. Furthermore, when the cells were modified with a lower concentration of choline analogues, 10 μg/ml, at which cell growth and macromolecular syntheses were not affected at all

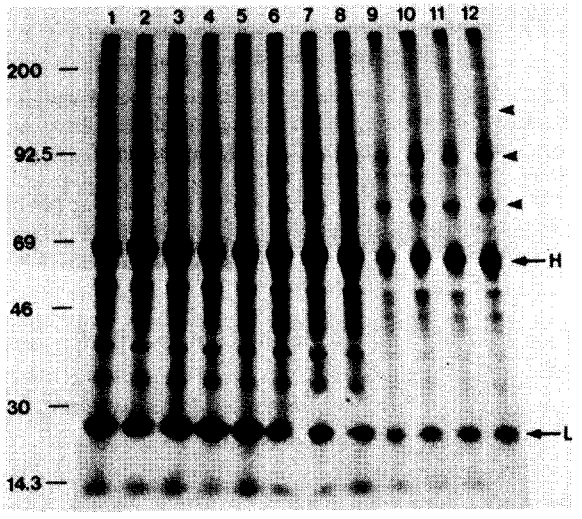

Fig. 2. Comparison of total proteins synthesized in MOPC-31C cells with modified phospholipids. Cells cultured with choline (lanes 1–4), DME (lanes 5–8) or ME (lanes 9–12) were pulse-labeled with [^{35}S]methionine for 10 min and chased as in Fig. 1. Cell lysates prepared from aliquots of the same volume withdrawn at 10 min (lanes 1, 5 and 9), 20 min (lanes 2, 6 and 10), 30 min (lanes 3, 7 and 11) and 45 min (lanes 4, 8 and 12) were reduced with dithiothreitol, alkylated with iodoacetamide and subjected to SDS-polyacrylamide 9–15% gradient gel electrophoresis. Arrows indicate the bands corresponding to H and L chains of IgG1. Arrow heads point to bands much less affected by the phospholipid modification. Molecular weight standards (values to the left, $\times 10^{-3}$) are: myosin (200000), phosphorylase *b* (92500), bovine serum albumin (69000), ovalbumin (46000), carbonic anhydrase (30000) and lysozyme (14300).

[21], a similar but small effect was still observed on the secretion of IgG1. Actually, half of the IgG1 was secreted about 1, 1.5 and 3 h after synthesis by the cells modified with 10 μg/ml of choline, DME and ME, respectively.

The amount of labeled IgG1 in ME-supplemented cells after 10 min pulse-labeling was about 30% of that in choline- or DME-supplemented cells (Fig. 1). However, the differences between ME-supplemented cells and choline- or DME-supplemented cells became smaller when the total protein syntheses were compared (data not shown). To examine whether the modification with ME affected the syntheses of various cellular proteins equally, the profiles on a SDS-polyacrylamide gel of total proteins synthesized de novo were compared among the three types of modified cell (Fig. 2). The quantities of proteins synthesized by ME-supplemented cells (lanes 9–12) were smaller in general than those of choline- or DME-supplemented cells (lanes 1–8). However, syntheses of some proteins (arrow heads) appeared to be much less affected in ME-supplemented cells than those of other proteins, showing that the synthesis of these proteins was less inhibited by the membrane modification with ME.

Intracellular transport of IgG1

The enzyme, endoglycosidase H, has been adopted as a useful tool for investigating the intracellular transport of various kinds of glycoprotein from the rough endoplasmic reticulum to the Golgi complex [3,30,31]. Immunoglobulins generally have aspargine-linked oligosaccharide chains, which are transferred to the H chain in the rough endoplasmic reticulum with a mannose-rich, endoglycosidase H-sensitive structure. Before export to the outside of cells, immunoglobulins pass through the Golgi complex, where the oligosaccharide chains are processed to have a complex-type structure which is no longer digested with endoglycosidase H. Thus, the extent to which immunoglobulins acquire resistance to endoglycosidase H can be a good measure of the fraction of immunoglobulins that has already reached or left the Golgi complex.

As described above, the secretion of IgG1 by MOPC-31C cells was markedly retarded by the phospholipid modification with DME or ME. To

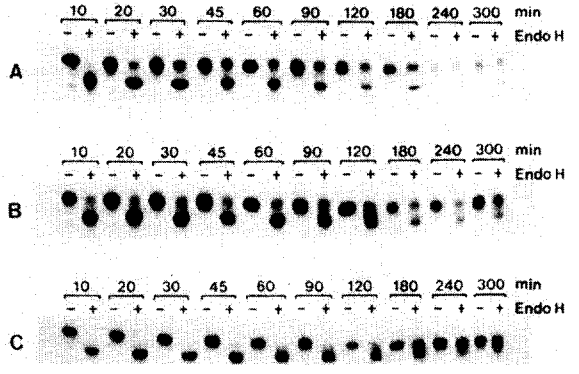

Fig. 3. Endoglycosidase H sensitivities of the IgG1 H chain in MOPC-31C cell lysates. Cells cultured with choline (A), DME (B) or ME (C) were pulse-labeled and chased as in Fig. 1. Intracellular IgG1 was isolated from aliquots taken at the times indicated, reduced with dithiothreitol and digested with endoglycosidase H for 14 h as described under Materials and Methods. Samples treated with (+) and without (−) endoglycosidase H were analyzed on SDS-polyacrylamide gels.

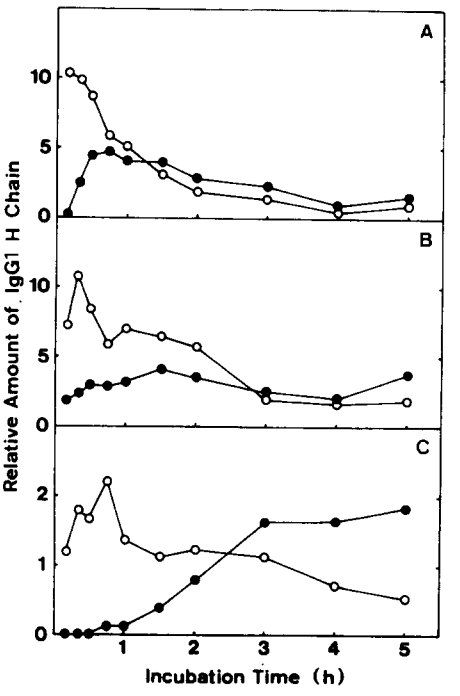

Fig. 4. Time courses for the IgG1 H chain to acquire endoglycosidase H resistance in MOPC-31C cells modified with choline (A), DME (B) or ME (C). The fluorograms in Fig. 3 were scanned with a densitometer and the relative amount of IgG1 H chain (arbitrary unit) sensitive (○) and resistant (●) to endoglycosidase H was plotted.

reveal the intracellular processes of IgG1 transport in these modified cells, IgG1 as isolated from cell lysates in a pulse-chase experiment (see Fig. 1) was subjected to endoglycosidase H digestion. Fig. 3 shows the fluorograms of SDS-polyacrylamide gels of the digests. In the control cells (Fig. 3A), the H chain of newly synthesized IgG1, which was completely endoglycosidase H-sensitive just after pulse-labeling (10 min), acquired the resistance very early during a chase and gradually disappeared on further incubation as a consequence of the secretion to medium. On the contrary, the rate at which IgG1 acquires endoglycosidase H resistance was appreciably lower in DME-supplemented cells (Fig. 3B) and was markedly decreased in ME-supplemented cells (Fig. 3C). It may be noted here that the H chain appeared to decrease in molecular weight by 5000–6000 when digested with endoglycosidase H, suggesting that this IgG1 molecule has two or three oligosaccharide chains in an H-chain monomer.

Densitometric scanning of these fluorograms provided time course profiles of intracellular movement of IgG1 (Fig. 4). Open and closed circles represent the relative amounts of IgG1 H chains sensitive and resistant to endoglycosidase H, respectively, at the times indicated. The half-times for IgG1 to acquire endoglycosidase H resistance were estimated from these figures to be 30–45 min, 1–1.5 h and 2–3 h for choline-, DME- and ME-supplemented cells, respectively. The intracellular IgG1 of ME-supplemented cells acquired endoglycosidase H resistance quite slowly and remained within the cells as an endoglycosidase H-resistant form even after 5 h. These observations imply that the transport of IgG1 from the rough endoplasmic reticulum to the Golgi complex was retarded by the modification with DME or ME, and the movement from the Golgi complex to the outside of cells was slowed down furthermore. The latter movement of IgG1 was remarkably inhibited by the alteration of membrane phospholipids with ME and, accordingly, IgG1 that had reached the Golgi complex appeared to be accumulated in the cells en route to the plasma membrane.

Morphological study

In order to investigate the structural alterations on phospholipid modifications of the organelles

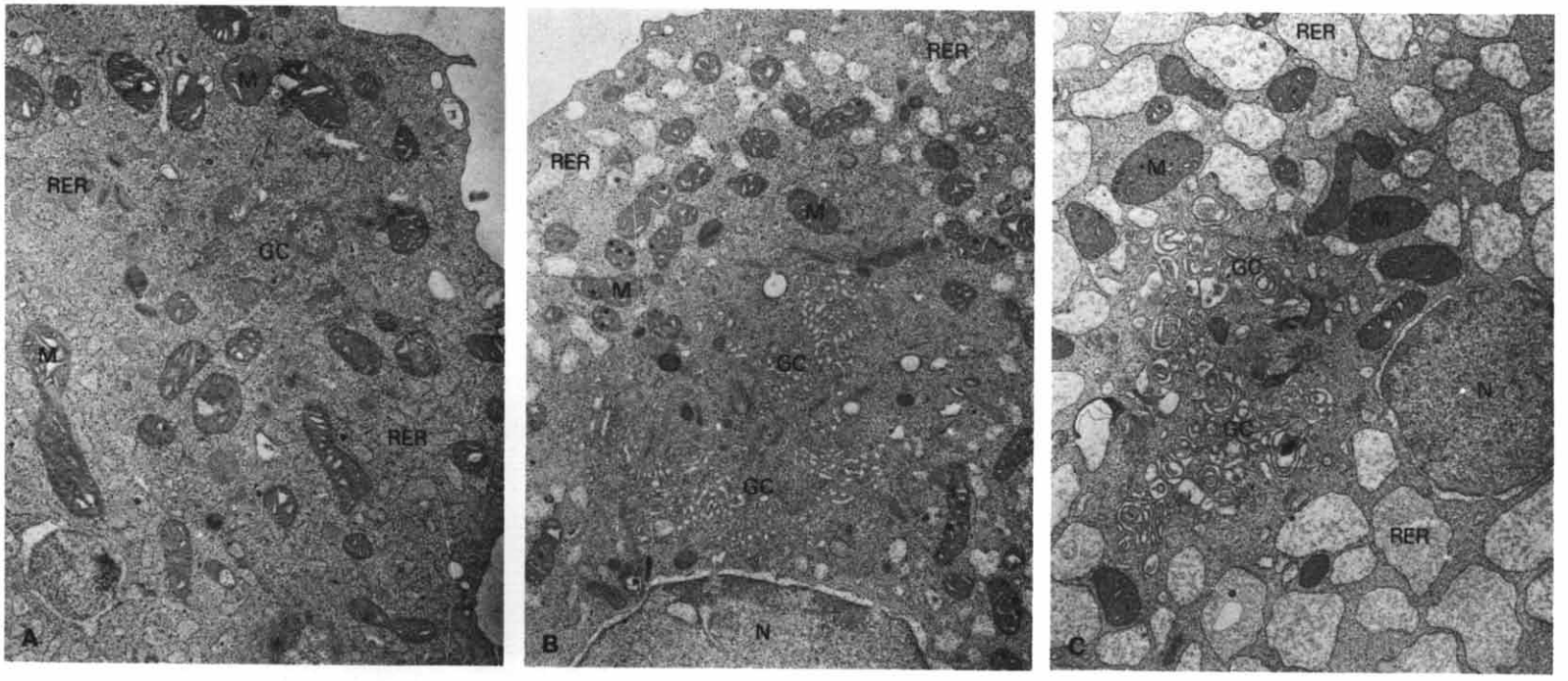

Fig. 5. Electron micrographs of MOPC-31C cells cultured with choline (A), DME (B) or ME (C). Details are described under Materials and Methods. RER, rough endoplasmic reticulum; GC, Golgi complex; M, mitochondria; N, nucleus. Magnifications are: ×9630 (A and B); ×7650 (C).

involved in IgG1 transport, the cells were cultured with choline, DME or ME for 3 days and then examined by electron microscopy (Fig. 5). As shown in Fig. 5C, phospholipid modification with ME caused curious deformation and dilation of the Golgi complex and the appearance of vacuolar structures probably derived from the rough endoplasmic reticulum. Smaller differences were observed in the structures of these organelles between DME-supplemented cells (Fig. 5B) and the control cells (Fig. 5A), though the Golgi complex still appeared to be dilated to some extent in DME-supplemented cells (Fig. 5B). Marked retardation of IgG1 transport in ME-supplemented cells described above may reflect the structural alteration of organelles as shown here.

Discussion

In the present study, we paid attention to the roles of phospholipids in the processes of intracellular transport and secretion of IgG1 by MOPC-31C cells and revealed that the modification of phospholipid composition with choline analogues retarded these processes (see Figs. 1 and 4). The modification with ME was proved to be much more effective than that with DME. We have previously shown [19] that the transport of glycoprotein of vesicular stomatitis virus from the endoplasmic reticulum to the plasma membrane in LM cells was also affected by the modification with choline analogues. The rate of glycoprotein transport was in the following order: DME-> choline-> ME-> ethanolamine-supplemented cells [19]. While DME was rather stimulatory in that case, the transport of viral glycoprotein was still slowed down by the modification with ME or ethanolamine. In the case of MOPC-31C cells, the phospholipid composition could not be altered by culturing with ethanolamine [21], but cells enriched with phosphatidylethanolamine are also expected to show retarded transport of IgG1. Thus, the transport of these two glycoproteins (IgG1 and viral glycoprotein) was similarly retarded by the modification with choline analogues, though they had different destinies, one to be secreted extracellularly and the other integrated in the plasma membrane. This implies that phospholipids play an important role in the 'membrane flow' from the rough endoplasmic reticulum to the plasma membrane.

The growth rate and the total protein synthesis of the cells supplemented with 40 μg/ml DME were essentially the same as those of the control cells. However, the treatment of MOPC-31C cells with 40 μg/ml ME affected the cell growth and the total protein synthesis to some extent. To minimize the pleiotropic effects of modification, if any, we carried out a similar experiment using a lower concentration of choline analogues, 10 μg/ml, at which cellular activities such as growth, protein synthesis and RNA synthesis were not affected at all [21]. The secretion of IgG1 was still slowed down by culturing with 10 μg/ml DME or ME, though the effect was smaller. Thus the modification with choline analogues can be considered to affect the transport of IgG1 directly, probably by modulating the processes of membrane flow. The condition employed in the present study, namely the treatment with 40 μg/ml ME, provided an almost extreme situation in vivo in which cells can be alive and made it possible to amplify the effect of phospholipid modification. Possibly, the decreased protein synthesis in the cells treated with 40 μg/ml ME is another direct effect of the modification of membrane phospholipids, as will be discussed later.

The choline analogues used in the present study are incorporated into the cells and the major membrane phospholipid, phosphatidylcholine, is substantially replaced by phosphatidyl-*N*,*N*'-dimethylethanolamine or phosphatidyl-*N*-monomethylethanolamine. As reported before [21], MOPC-31C cells have the metabolic pathway converting phosphatidylethanolamine to phosphatidylcholine by sequential methylation via transient intermediates, phosphatidyl-*N*-monomethylethanolamine and phosphatidyl-*N*,*N*'-dimethylethanolamine [32]. The modification with DME or ME in the present study thus can be regarded as an enrichment with the intermediates of this methylation reaction. Hirata and Axelrod have proposed that the phospholipid methylation has an important role in various functions of biomembranes such as responses to hormones or mitogens, since the transfer of methyl groups of phosphatidylethanolamine alters the microenvironments in a membrane to a more fluid or mobile state [15]. In this regard, it is

very interesting that the enrichment of such intermediates in the cells in place of phosphatidylcholine retarded the intracellular transport and secretion of IgG1. In fact the rate of IgG1 transport was in the order: choline-> DME-> ME-supplemented cells. However, the modification with choline analogues when supplemented exogenously does not necessarily alter the fluidity of membranes, because the cells are likely to vary in fatty acid composition or cholesterol content to compensate for the fluidity change produced by the modification of phospholipid bases [33]. It remains to be elucidated whether the microviscosity of the membranes of MOPC-31C cells is actually altered by the present phospholipid modification or not. It may be worthwhile to mention here that the activities of phospholipid methylation are remarkably enhanced in cells modified with DME or ME [21], mainly because of the increase of endogenous substrates. The relationships between such enhanced methylation activities and the decreased rates of intracellular transport and secretion of IgG1 are unclear at present.

The decreased activities of other membrane enzymes such as processing enzymes of oligosaccharide chains may also cause the retardation of IgG1 transport in the cells with modified phospholipids. Inhibition of glycosylation by tunicamycin has been reported to have various effects on the secretion of immunoglobulins. The secretion of IgA, IgE or IgM was greatly inhibited, whereas that of IgG was little affected [5,7]. In the case of MOPC-31C cells, tunicamycin also appeared to have little effect on the rate of IgG1 secretion (unpublished observations). Therefore, it is unlikely that oligosaccharide-processing enzymes produce any retardation of IgG1 secretion.

As described above, the modification with ME lowered the level of total protein synthesis by MOPC-31C cells. However, the effect of ME supplementation was not equal for all proteins as shown in Fig. 2. Syntheses of some proteins were much less affected than that of IgG1 by the modification with ME. It is well-established that proteins destined to be secreted, transported to lysosomes or integrated into plasma membranes are synthesized on membrane-bound ribosomes, whereas cytoplasmic proteins and most of mitochondrial proteins are synthesized on free ribosomes [34–36]. Then it may be reasonable to assume that the modification of membrane phospholipid primarily affect the former protein syntheses. It is not likely that all syntheses on free ribosomes were much less affected by ME supplementation than those on membrane-bound ribosomes, since only a limited number of proteins appeared to be synthesized in comparable amounts as in choline- or DME-supplemented cells. Further investigation must be carried out to reveal the characteristics of these less affected proteins. Nevertheless, the observation that the phospholipid modification significantly inhibited the synthesis of IgG1, leaving those of some other proteins less affected, suggests an important role of membrane phospholipids in the first step of biogenesis of secretory proteins, that is, the translocation across the membrane of the rough endoplasmic reticulum. Studies using cell-free systems are expected to be fruitful for investigating the roles of phospholipids in this earliest process and are now in progress.

One also has to be careful in analyzing the intracellular transport of glycoproteins on the criterion of endoglycosidase H sensitivities. Rothman and his co-worker [37,38] have recently pointed out that the Golgi cisternae may be divided into two functionally distinct organelles. According to their proposal, the glycosyltransferases which process oligosaccharide chains to be endoglycosidase H-resistant are located in the *trans* portion of the Golgi cisternae and a glycoprotein just transported from the endoplasmic reticulum to the *cis* portion of the Golgi stack is still sensitive to endoglycosidase H. It is possible that endoglycosidase H-sensitive fraction of IgG1 in the present study contained IgG1 that had already reached the Golgi complex but had not yet been processed to bear complex-type oligosaccharide chains. Of course, such a possibility does not affect our conclusion on the retardation of intracellular transport of IgG1 and the roles of membrane phospholipids in the processes of IgG1 transport are still of great importance.

In summary, membrane phospholipids were found to be closely involved in intracellular transport and secretion of IgG1. The modification of phospholipid composition with DME or ME caused marked retardation of these processes. Sim-

ilar approaches with cultured cells, whose membrane phospholipids can be easily modified, will be useful for elucidating the roles of phospholipids in various other functions of biomembranes.

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