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CHANGES IN FATTY ACYL CHAINS OF PHOSPHOLIPIDS INDUCED BY INTERFERON IN MOUSE SARCOMA S-180 CELLS

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Summary: S-180 mouse sarcoma cells exhibit antiviral and antiproliferative responses to mouse β interferon. The composition of cellular phospholipids was specifically altered as a result of treatment with interferon. The unsaturated fatty acid content of all of the major phospholipids was decreased, resulting in an increase in the relative proportions of the saturated acyl side chains. These changes can be prevented by anti-interferon antibody and they are not observed with human interferon.

INTRODUCTION: Although interferon was originally described and characterized as an antiviral substance, evidence accumulated over recent years has suggested that this glycoprotein can also exert profound pleiotropic effects on homologous cells both *in vivo* and *in vitro* (1,2). In various tissue culture systems interferons have been shown to inhibit the proliferation of normal as well as transformed cells (3). Interferon treatment also results in major structural and functional modifications of cells (4,5). Plasma membranes have been implicated as possible targets for both the antiviral and antiproliferative actions of interferon (6-8). The inhibition of virus maturation and release (9,10) in interferon-treated cells is possibly a reflection of altered membrane properties DNA, RNA and protein synthesis are only slightly reduced in interferon-treated cells; these are presumably not sufficiently suppressed to account for the antiproliferative effects (6).

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Recent studies have therefore focused attention on possible alterations of cellular architecture to explain the antiproliferative effects of interferon treatment. In addition to major changes that result in increased surface area, volume and mass, there is reduced cell motility (4,5). These studies have implicated the involvement of plasma membranes in the following changes. In interferon-treated cells: a) the arrangement of fibronectin is changed markedly; b) the ability of cells to redistribute concanavalin A receptors is inhibited (11); and c) the rigidity of plasma membrane bilayers as judged by ESR spectroscopy is markedly increased (12). The latter two observations could conceivably result from alterations in the fluidity of membrane lipids. Fatty acyl side chains of membrane phospholipids are the principal structural components regulating membrane transitions. Therefore, compositional changes in the hydrocarbon side chains of phospholipids could be one of the molecular alterations underlying the interferon-induced morphological manifestations. Here we report that in interferon-treated cells the unsaturated fatty acid content of all major phospholipids is reduced, resulting in a membrane in which the ratio of saturated to unsaturated fatty acids is increased. These lipid bilayers would be expected to be considerably more rigid.

MATERIALS AND METHODS: Cells. S-180 Mouse sarcoma cells were purchased from American Type Culture Collection (cat. No. C.CL.8). These cells were passaged in minimum essential medium with 5% fetal calf serum or newborn calf serum (Gibco) at a split-ratio of 1:6. Cells were grown to confluency in the seeded medium without change.

Interferon. Mouse fibroblast interferon [(β) 2 x 10^7 units/mg of protein], prepared from mouse C-243 cells, was a generous gift from Dr. Ion Gresser. Partially purified human lymphoblastoid interferon with a specific activity of 0.58 x 10^6 units/mg of protein was from Dr. Norman Finter, Wellcome Research Laboratories, Beckenham, England.

The antiviral and anticellular response of S-180 cells to mouse β interferon. The antiviral response was measured by a plaque reduction assay (13). For measuring the anticellular activity, cells were seeded at low density (4 x 10^4 cells/5 cm petri dish) and the number of cells was counted every day until the control culture reached saturation. With 200 units of interferon/ml of medium, S-180 cells failed to divide beyond one growth cycle. Very few mitotic cells were seen after the 2nd day, and cells began to detach after the third day.

Lipid extraction and chromatography. Cell monolayers were washed with phosphate buffered saline 3-times. 5 ml methanol at dry ice temperature were added to the monolayer; after 2 min, the cells were quickly scraped with a rubber policeman and transferred with the methanol to 10 ml of chloroform. 250 micrograms of dinona decanoyl Lα-lecithin (Supelco, Bellefonte, PA) were

added to the lipid extract as an internal standard. After 24 hrs in the freezer the insoluble precipitate was removed by filtration, and the lipid extract was partitioned and washed, by the method of Folch et al. (14). The chloroform layer was evaporated to dryness, and the lipids were chromatographed on silica gel G plates (Brinkman) and developed first with chloroform/methanol/water (65:25:4, v/v). The plates were dried and dessicated for 3 hrs in a vacuum dessicator and run in the second dimension with the solvent system, tetrahydrofuran/methylal/methanol/ water (10:6:4:1, v/v). The lipids were localized with iodine vapour and areas of the chromatogram corresponding to phosphatidyl choline, phosphatidylethanolamine and phosphatidylinositol were scraped into methanol and extracted five-times with 3 ml of methanol. The methanol was evaporated and the phospholipids were hydrolyzed with 0.8 N anhydrous methanolic HCl at 80°C for 8 hrs in a sealed tube. Fatty acid methyl esters were extracted into hexane (5 x 3 mls), evaporated to dryness, redissolved in a suitable volume and injected into the gas chromatograph.

Lipid-phosphorous was determined by the method of Bartlett (15).

Gas-Liquid chromatography of fatty acid methyl esters. A Tracor 560 gas chromatograph fitted with a flame ionization detector was used for the analysis. A 6' x ¼" glass column (2 mm inner diameter) was packed with 10% Silar 10C on 100/120 Gas Chrom R (Applied Science Laboratories, State College, PA). Nitrogen at 15 ml/min was used as the carrier gas. Fatty acids were identified from their retention times by running known standards, and quantitated with respect to the internal standard.

RESULTS AND DISCUSSION: Cultured cells generally utilize the fatty acids of the serum lipids present in the medium for synthesis of the acyl groups of their phospholipids rather than synthesizing fatty acids de novo. Despite this preference for the fatty acids present in the medium, the various individual phospholipids exhibit a spectrum of fatty acids which is characteristic of cellular metabolism rather than simply reflecting their order of abundance in the medium (16). In S-180 cells grown in fetal calf serum, either stearate or palmitate was the major saturated fatty acid and oleate was the major unsaturated fatty acid in all of the phospholipids analyzed (Table 1). Although significant quantities of linoleate are present in phosphatidylcholine and phosphatidyl ethanolamine, this fatty acid is notably absent from phosphatidylinositol. Treatment with interferon shifts the fatty acid composition of phospholipids predominantly towards a more saturated state. This trend towards increased saturation is manifest regardless of the nature of the phospholipid examined. Thus, a substantial reduction of linoleate is apparent in phosphatidylcholine and in phosphatidylethanolamine (Tables I and III). However, in phosphatidylinositol, which contains no detectable linoleate, oleate is the fatty acid that is reduced substantially (Tables I and III). The level of oleate is not signi-

Table I

Fatty Acid Composition of Phospholipids* of Control and Interferon-Treated S-180 Cells Grown in Fetal Calf Serum

Phospholipid	Treatment	Fatty acid (expressed as percentage of total)						
		16:0	18:0	18:1	18:2	20:4	UnId‡	DBI®
Phosphatidylcholine	CON	22.6	13.9	35.4	20.4	1.6	5.8	82.6
	IFN	25.0	17.2	35.7	14.8	0.8	6.3	68.5
Phosphatidylethanolamine	CON	10.7	31.4	30.5	16.1	7.4	3.7	92.3
	IFN	13.1	47.2	28.9	6.5	0.5	3.5	43.9
Phosphatidylinositol	CON	14.1	44.0	15.4	-	15.1	11.3	75.8
	IFN	16.1	52.3	10.0	-	8.6	12.9	44.4

® DBI = Double Bond Index (number of double bonds x mass %)

Cells were grown in MEM with 5% fetal calf serum. The medium was changed at confluency to MEM with 2% fetal calf serum. 15 x 10^6 cells received mouse β interferon at 1000 units/ml. After 12 hrs the medium was aspirated and the monolayer was processed for lipid analyses as described in Methods. Integrated peak areas of all fatty acids on the chromatogram are taken as total (100%).

*Lipid phosphorous analysis shows the following composition for phospholipids: Phosphatidylcholine 42%, phosphatidylethanolamine 41.6% and phosphatidylinositol 8% of total phospholipids.

‡ Unidentified

ficantly reduced in either phosphatidylcholine or phosphatidylethanolamine when expressed as a percentage of the total fatty acids (Table I). However, reductions are noted in individual chromatograms (Fig. 1). Arachidonate is consistently reduced in all phospholipid classes after interferon treatment (Tables I and III).

The overall percentage of saturated fatty acids would be expected to increase when the quantities of unsaturated fatty acids in phospholipids are reduced. However, this increase in percentage composition may not necessarily represent a net increase in the quantity of saturated fatty acids when normalized to the cell protein. The double bond index (sum of the number of double bonds x mass percent of the compound) probably reflects changes in the overall acyl chain saturation in a more meaningful way (16). Substantial changes in double bond index are indeed apparent in all three phospholipids (Table I).

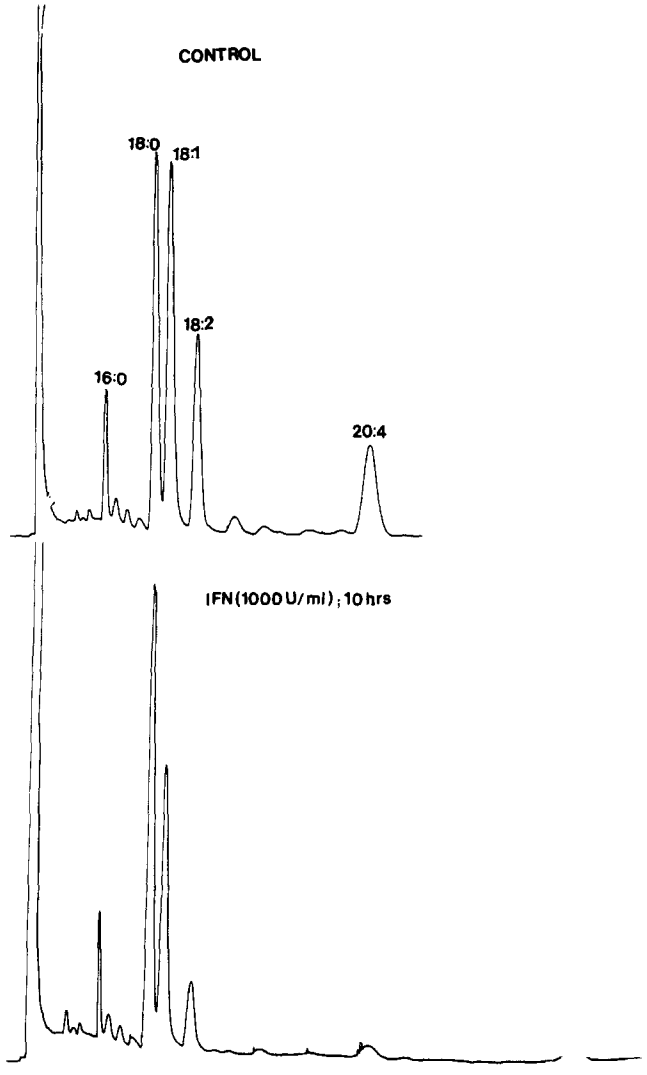

Figure 1. Gas chromatogram of phosphatidylethanolamine from control and interferon-treated S-180 cells.
Fatty acid methyl esters were run isothermally at 200°C, as described in Materials and Methods. These samples were processed without added internal standard.

These changes are reduced upon addition of antibody against β interferon, which also neutralizes the antiviral activity of interferon. The changes are not observed when human lymphoblastoid interferon (2000 U/ml) is substituted for mouse interferon in the experiments described using S-180 cells.

Analysis of the palmitate content of the three phospholipid classes showed that there is no change in this fatty acid when its concentration is normalized

Table II

Molar ratio of palmitic acid ($\frac{\text{moles of lipid phosphorous}}{\text{moles of C16:0 in the lipid}}$) in major phospholipids of S-180-cells before and after treatment with β IFN*

Treatment	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Phosphatidyl-inositol
Control	4.8 ± 0.6	10.1 ± 1.2	7.6 ± 0.9
Interferon	5.2 ± 0.7	10.2 ± 1.5	7.1 ± 0.8

* 100 U/ml for 12 hrs.

against lipid phosphorous (Table II). The quantities of other fatty acids in each phospholipid class could therefore be estimated relative to the palmitate content (Table III). These data show that the conclusions drawn from percentage composition are valid also on the basis of net changes in the concentrations in fatty acids with respect to the reduction in unsaturated fatty acids.

Table III

Specific reduction in the concentration of unsaturated fatty acids of S-180 cellular phospholipids after interferon treatment*.

Fatty acid	Treatment	Phospholipid		
		Phosphatidyl ethanolamine	Phosphatidyl choline	Phosphatidyl inositol
16:0	Con	1	1	1
	IFN	1	1	1
18:0	Con	2.9	0.6	3.1
	IFN	3.5	0.7	3.2
18:1	Con	2.8	1.6	1.1
	IFN	2.2	1.4	0.6
18:2	Con	1.5	0.9	-
	IFN	0.5	0.6	-
20:4	Con	0.7	0.07	1.1
	IFN	0.03	0.03	0.5
Total unsaturated / Total Saturated	Con	1.2	1.6	0.6
	IFN	0.6	1.1	0.2

* Since the palmitate content was found to be unchanged as a result of interferon treatment (Table II), the concentrations of individual fatty acids in each lipid were normalized with respect to C16:0 in each chromatogram. The data therefore represent the number of moles of each of the listed fatty acids present per mole of palmitate in the indicated lipid.

However, there does not appear to be a net increase in the levels of saturated fatty acids, with the possible exception of stearate in phosphatidylethanolamine (Table I). The ratios of total unsaturated to total saturated fatty acids show that phosphatidylethanolamine and phosphatidylinositol are noticeably reduced in their unsaturated fatty acid content.

When the same cells were grown in newborn calf serum, the fatty acid composition of all phospholipids changed in one important respect. Due to the very small quantities of linoleate and arachidonate in this lot of serum, all phospholipids contained only trace amounts of these fatty acids. Despite this change in the fatty acyl pattern, the overall composition of the various phospholipids yielded ratios for saturated fatty acids to unsaturated fatty acids which were comparable to those given in Table III. Their double bond indices were also similar to those given in Table II (data not given). These observations suggest that the cell regulates its unsaturated fatty acid content to an optimal value by increasing the concentrations of the available unsaturated fatty acids. In this case oleate was increased in amounts large enough to compensate for the absence of linoleate, resulting in double bond indices comparable to those of cells grown in fetal calf serum. When these cells were treated with interferon, the double bound index and the saturated to unsaturated ratios shifted in the same direction as shown in Tables I and III, except that the reductions were less pronounced. In these experiments oleate, the only unsaturated fatty acid present, decreased as a result of interferon treatment.

The lipid bilayer in biological membranes must have a finite percentage of unsaturated fatty acids to achieve given degrees of fluid structure appropriate for required functions. Some vital functions that have been shown to be affected by specific changes in the unsaturated fatty acid composition of membranes include sugar transport (17), cation transport in mitochondria (18) and coupling of ATP synthesis to cellular respiration (19). The changes induced by interferon could thus conceivably lead to structural alterations that could interfere with energy metabolism in a manner that could lead to "anticellular" actions. Such

changes might also play a role in the interferon-induced altered membrane architecture (4,5), possibly resulting in inhibition of virus release (10). The changes could certainly explain the increased rigidity of plasma membranes observed by ESR spectroscopy after interferon treatment (12).

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