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EFFECT OF GLYCOLIPIDS DETECTABLE IN TRANSFORMED HUMAN CELLS ON INTERFERON ACTIVITIES

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SUMMARY. Human transformed cell lines, RSa, RSb, and IF^r, were analyzed for their glycolipid composition. Ganglioside GM_2, GM_3, and 4 neutral glycolipids were identified as the component of these 3 cell lines. Antiviral action of human interferon was neutralized by pretreatment with GM_3 or GM_2. Lactosylceramide also neutralized antiviral action of human interferon. Thus, these glycolipids are considered to be the components of the receptor for interferon in these cells. However, when GM_3 or ganglioside mixture was tested for the anticellular action of human interferon, they showed no inhibitory action on the growth suppressive activity of interferon.

Some glycolipids are known to constitute cell surface receptors for various proteins and to participate in the transmission of membrane-bound information (1, 2). The receptor for interferon has been attributed to gangliosides, especially GM_2 in the case of mouse cells (3, 4). Concerning receptors on human cells, Vengris et al. (5) examined binding activities of ganglioside mixture and several ganglioside including GM_1 and GM_2. They found the antiviral action of human fibroblast interferon to be neutralized by such gangliosides without particular ganglioside specificity. Their results suggested that such membrane gangliosides may play a role in the antiviral action of human interferon.

We have reported on the human cell lines, RSa and RSb, which are highly sensitive to the anticellular action of human interferon (6, 7). Therefore, these cells provide a good experimental tool to analyze receptors for interferon on the plasma membrane. We report herein the glycolipids composition of these cells and interaction of such glycolipids and human interferons on these cells.

MATERIALS AND METHODS

Cells. Human transformed cells, RSa, RSb, and IF^r, were used as before (6, 7, 8). The cells were seeded in either 6-cm or

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15-cm diameter plastic dishes (Falcon) by using Eagle's minimum essential medium (MEM) supplemented with 10% calf serum and cultured in the CO_2 incubator at 37°.

Analysis and identification of glycolipds. Total cellular lipids were extracted from the cell homogenate (10^8 cells) with chloroform and methanol (9). Residues were extracted successively with 20 volumes of chloroform-methanol (2/1 and 1/1, v/v). Pooled extracts were separated into neutral and acidic fractions by DEAE-Sephadex column chromatography (10). After dialysis, each fraction was evaporated to dryness and the neutral fraction was acetylated with pyridine and acetic acid (1/1, v/v) at 60° for 6 h. Acetylated glycolipid fraction was obtained by Florisil column chromatography (11). Acetylated glycolipids were separated by thin layer chromatography (TLC) and analyzed by gas liquid chromatography (12), or in some cases, these acetylated glycolipids were further N-p-nitrobenzoylated and subjected to analysis by high-performance liquid chromatography (13). Acidic fraction was separated by TLC and estimated by periodate-resorcinol reagent (14).

TLC was performed with precoated silica gel 60 plate (E. Merck, Darmstadt). Solvent systems were chloroform-methanol-water (65/25/4 or 60/35/8, v/v/v) for glycolipids, chloroform-methanol-2.5 N ammonia (65/35/8, v/v/v) or chloroform-methanol-0.2 % $CaCl_2$ (55/45/10, v/v/v) for acidic glycolipids, and chloroform-methanol (97/3, v/v) for acetylated glycolipids. Neutral glycolipids were detected with the anthrone-sulfuric acid reagent (15) and gangliosides with the resorcinol reagent (16).

Glycolipid preparation. Ganglioside mixture and GM_1 were from bovine brain, and GM_2 from brain of a patient with Tay-Sachs' disease (17). GM3 was from dog erythrocytes (18). Glucosyl- and galactosyl-ceramide were obtained from the spleen of a patient with Gaucher's disease and from pig brain, respectively (19). Lactosylceramide, trihexosylceramide and globoside were obtained from human erythrocytes (20).

Virus. Vesicular stomatitis virus (VSV) of Indiana serotype was used for the assay of interferon. VSV was grown in the RSa cells and aliquots of virus suspension were kept at -75°.

Test for suppression of antiviral action of interferon by glycolipids. We followed in general the method reported by Vengris et al. (5). Equal volumes of interferon preparation and glycolipid suspension diluted with serum-free MEM were mixed and incubated at 37° for 1 h. Then interferon or glycolipid or their mixture was applied to RSa cell sheets in 6-cm diameter plastic plates. After incubation for 18 to 20 h, the cells were washed once with serum-free MEM, and then challenged with VSV. Culture fluids were harvested about 16 h after infection, and $TCLD_{50}$ was measured by using RSa cells in Linbro multiwell dishes.

Interferons. Human leukocyte interferon (Le-IF) was kindly supplied by Dr. K. Cantell, Central Public Health Laboratory, Helsinki. It had a titer of 10^6 units/ml and specific activity was 7.5×10^5 units/mg protein. Human fibroblast interferon (F-IF) was supplied by Dr. R. Skoda, Rentschler Laboratory, Laupheim, Germany, and by the National Institutes of Health, U.S.A. It contained 10^6 units/ml.

Assay of interferon. Le-IF and F-IF were assayed by the inhibition of VSV-induced cytopathic effect on RSa cells. Titer

of IF was expresed as a reference unit/ml by comparing with the National Institutes of Health human interferon reference standard.

RESULTS AND DISCUSSION

Glycolipid composition of human transformed cells.

Glycolipid patterns of three cell lines showed no qualitative difference on the TLC plate. This was also confirmed by quantitative analysis as shown in Table 1, although there was some variation in the analytical values. All the cell lines contained glucosylceramide, trihexosylceramide, globoside, GM_3, and GM_2. These glycolipids were identified by TLC analysis, their sugar composition, and susceptibility to the neuraminidase from Cl. perfringens. The presence of a relatively high concentration of GM_2 is remarkable. Concerning the presence of GM_2, our results conform with that reported by Hakomori (21) and by Brady and Fishman (22) on human fibroblasts transformed by SV40.

Effect of glycolipids on the antiviral action of human interferons.

Ganglioside GM_2 (100~200 μg/ml) was first tested in our system for their capacity to neutralize antiviral action of Le-IF (100 units/ml). GM_2 neutralized interferon action when preincubated in vitro (data not shown), as already reported by Vengris et al. (5). Next, inhibitory effect of GM_3 on Le-IF was tested (Table 2). GM_3 showed almost the same degree of inhibitory action on Le-IF as GM_2. When F-IF was used instead of Le-IF, more pronounced effect was demonstrated (Table 2). Thus, the terminal N-acetylgalactosaminyl residue seems to be not essential for the interaction between human interferon and gangliosides. Since GM_3 was detected in human diploid fibroblasts (23, 24) and also in our transformed cells, this ganglioside may play an important role as a part of the receptor for interferon on the plasma membrane.

To ascertain whether terminal sialic acid residue is necessary for the interaction between interferon and glycolipids, samples devoid of sialic acid residue were tested in the next experiments. Trihexosylceramide and glycosylceramide did not significantly neutralize the antiviral action of Le-IF. However, lactosylceramide showed inhibition of interferon action (Table 3), though not so remarkable as in the case of GM_2 or GM_3. Inhibitory action of lactosylceramide was also detected against F-IF. For comparison N-acetylneuraminyl lactose (200 μg/ml, Calbiochem) was tested for the inhibition of antiviral action of Le-IF and almost no effect

Table 1. Glycolipid composition of transformed human fibroblasts

Glycolipids	nmol/g protein		
	RSa	RSb	IF^r
Glucosylceramide	1,425	964	1,439
Lactosylceramide	367	231	328
Trihexosylceramide	236	162	194
Globoside	48	34	38
GM3	585	728	655
GM2	735	1,027	765

was demonstrated. Thus, ceramide portion may be necessary for interaction with IF. On the other hand, galactosylcermide (400 μg/ml) showed a slight, if any, interaction with Le-IF. As Besancon and Ankel (4) pointed out, shortening of the saccharide portion may prevent inhibition of IF and IF interacts with the carbohydrate moiety of glycolipids.

Effect of gangliosides on the anticellular action of human interferon.

Effect of ganglioside mixture (200 μg/ml) on the anticellular action of Le-IF was examined (Fig. 1a). In RSa cells, ganglioside mixture did not significantly affect their growth rate. However, when the cells were treated with a mixture of interferon and gangliosides, growth of the cells was rather more suppressed than by single treatment with interferon. This result was also confirmed in RSb cells. The same kind of experiment was performed by using GM_3 and Le-IF (Fig. 1b). In this case, combined treatment with GM_3 and Le-IF did not influence the anticellular action of Le-IF.

Mechanisms of cell growth suppressing acton of interferon is not yet clear. Inhibitors of antiviral action of interferon such as cycloheximide and ouabain did not block the anticellular action of interferon (25, 26). On the other hand, hormone receptor complexes are postulated to bind to another membrane structure such as adenylate cyclase (27). Thus, it is conceivable that interferon

Table 2. Effect of ganglioside GM3 on the antiviral action of Le-IF and F-IF

Le-IF* (units/ml)	GM3* (μg/ml)	VSV titer (log $TCID_{50}$/0.2 ml)	F-IF* (units/ml)	GM3* (μg/ml)	VSV titer (log $TCID_{50}$/0.2 ml)
0	0	7.7	0	0	7.7
0	200	7.2	0	200	7.7
100	0	4.7	100	0	4.7
100	200	7.0	100	200	8.0
100	100	6.2	100	100	7.5 ≤
100	50	5.5	100	50	7.2 ≤
50	0	5.2	50	0	6.0
50	200	7.0	50	200	8.0
50	100	6.2	50	100	7.5 ≤
50	50	6.0	50	50	7.5 ≤

* Interferon and GM3 in Eagle's MEM were preincubated for 1 h at 37°, and then added to the RSa cell sheets as described in Materials and Methods.

Table 3. Effect of lactosylceramide on the antiviral action of Le-IF and F-IF

Le-IF (units/ml)	Lactosylceramide (μg/ml)	VSV titer (log $TCID_{50}$/0.2 ml)	F-IF (units/ml)	Lactosylceramide (μg/ml)	VSV titer (log $TCID_{50}$/0.2 ml)
0	0	7.5	0	0	7.5
0	200	7.2	0	200	7.7
100	0	5.2	100	0	5.0
100	200	7.2	100	200	6.2
100	100	6.5	100	100	6.2
100	50	6.5	100	50	5.5
50	0	5.5	50	0	5.7
50	200	7.0	50	200	$6.5 \leq$
50	100	$6.7 \leq$	50	100	6.7
50	50	7.0	50	50	5.7

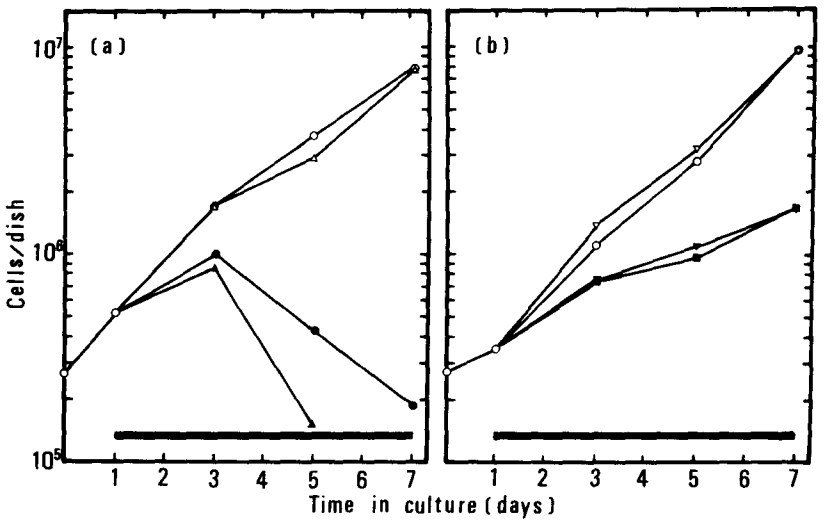

Figure 1. Effect of gangliosides and Le-IF on the growth of RSa cells. (a) ○—○, control cells; △—△, 200 μg/ml of ganglioside mixture; ●—●, 500 units/ml of Le-IF; ▲—▲, 500 units/ml of Le-IF and 200 μg/ml of ganglioside mixture. (b) ○—○, control cells; ▽—▽, 200 μg/ml of GM3; ■—■, 200 units/ml of Le-IF; ▼—▼, 200 units/ml of Le-IF and 200 μg/ml of GM3. ▬▬, period of treatment.

exhibits its anticellular action even after combining with ganglioside mixture or GM_3 despite inhibition of its antiviral action.

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REFERENCES

1. Fishman, P. H. and Brady, R. D. (1976) Science 194, 906-915.
2. Critchley, D. R., and Vicker, M. G. (1977) Dynamic Aspects of Cell Surface Organization, pp. 307-370, North-Holland Publishing Co., Amsterdam.
3. Besançon, F., and Ankel, H. (1974) Nature (London), 252, 478-480.
4. Besançon, F., Ankel, H. and Basu, S. (1976) Nature (London), 259, 576-578.
5. Vengris, V. E., Reynolds, F. H., Jr., Hollenberg, M. D., and Pitha, P. M. (1976) Virology 72, 486-493.
6. Kuwata, T., Oda, T., Sekiya, S., and Morinaga, N. (1976) J. Natl. Cancer Inst. 56, 919-926.
7. Kuwata, T., Fuse, A., and Morinaga, N. (1976) J. Gen. Virol. 33, 7-15.
8. Fuse, A., and Kuwata, T. (1977) J. Natl. Cancer Inst. 58, 891-896.
9. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911-917.

10. Lendeen, R. W., Yu, R. K., and Eng, L. F. (1973) J. Neurochem. 21, 829-839.
11. Saito, T. and Hakomori, S. (1971) J. Lipid Res. 8, 257-259.
12. Vance, D. E. and Sweeley, C. C. (1967) J. Lipid Res. 8, 621-630.
13. Yamasaki, T., Suzuki, A., Handa, S., Hamanaka, S., and Yamakawa, T. (1978) Proc. Jpn. Conf. Biochem. Lipid. 20, 335-338 (in Japanese).
14. Jourdian, G. W., Dean, L., and Roseman, S. (1971) J. Biol. Chem. 246, 430-435.
15. Roe, C. H. (1955) J. Biol. Chem. 211, 335-343.
16. Svennerholm (1957) Biochim. Biophys. Acta 24, 604-611.
17. Momoi, T., Ando, S., and Nagai, Y. (1976) Biochim. Biophys. Acta 441, 448-497.
18. Yasue, S., Handa, S., Miyagawa, S., Inoue, J., Hasegawa, A., and Yamakawa, T. (1978) J. Biochem. (Tokyo), 83, 1101-1107.
19. Radin, N. S. (1976) J. Lipid Res. 17, 290-293.
20. Ando, S. and Yamakawa, T. (1973) J. Biochem. (Tokyo), 73, 387-396.
21. Hakomori, S. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 1741-1747.
22. Brady, R. O. and Fishman, P. H. (1974) Biochim. Biophys. Acta 355, 121-148.
23. Dawson, G., Matalon, R., and Dorfman, A. (1972) J. Biol. Chem. 247, 5944-5950.
24. Fishman, P. H., Moss, J., and Manganiello, V. C. (1977) Biochemistry 16, 1871-1875.
25. Kuwata, T., Fuse, A. and Morinaga, N. (1977) J. Gen. Virol. 34, 537-540.
26. Kuwata, T., Fuse, A. and Morinaga, N. (1977) J. Gen. Virol. 37, 195-198.
27. Siegel, M. I. and Cuatrecasas, P. (1975) Cellular Membranes and Tumor Cell, pp. 13-140, Williams and Willkins Co., Baltimore.