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EFFECT OF GLYCOLIPIDS AND GLYCOPHORIN ON THE ACTIVITY OF HUMAN INTERFERON-β AND -γ

AKIRA FUSE[1], SHIZUO HANDA[2] and TSUGUO KUWATA[1]

[1] *Department of Microbiology, School of Medicine, Chiba University, 280 Chiba; and* [2] *Department of Biochemistry, Tokyo Medical and Dental University, 113 Tokyo, Japan*

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We have studied neutralization of the antiviral activity of human fibroblast (IFN-β) and immune interferon (IFN-γ) by incubation with glycolipids (including the gangliosides GM1, GM2 and GM3, as well as various other glycolipids) and with the sialoglycoprotein, glycophorin. When 100 units/ml of IFN-β were preincubated with 30–80 μM of the gangliosides, all antiviral activity was abolished. Similarly, 120 μM of glycophorin completely reversed the antiviral activity of 100 units/ml of IFN-β. Glycolipids containing more than two sugars also showed moderate inhibitory effects. GM2 at a concentration of 200 μM almost completely inhibited the antiviral activity of 100 units/ml of IFN-γ, but GM1, GM3 and glycophorin had only a moderate inhibitory effect. These results suggest that the terminal *N*-acetylneuraminic acid (NANA) residues of gangliosides and of glycophorin play an important role in the inhibition of IFN-β, and that they may be similarly involved in the inhibition of IFN-γ.

interferon, types β and γ ganglioside glycophorin

INTRODUCTION

The existence of specific cell membrane receptors for peptide hormones, toxins, mitogens and lectins has been postulated. Several lines of evidence suggest that there also exists a cell membrane receptor system which specifically binds interferon (IFN) and subsequently activates the metabolic steps which lead to the variety of IFN effects on cells. Such a receptor system would not require internalization of IFN. Thus IFN retains its activity even if bound to Sepharose beads which are not taken up by cells [3], and cells induced to produce IFN do not develop an antiviral state if antibodies to IFN are present [18]. Furthermore, antibodies against mouse/human hybrid cells which carry human chromosome 21 have been shown to block the action of human IFN [15], implying that the antibodies are directed against the chromosome 21-coded receptor system for IFN.

The antiviral action of IFN can be inhibited by preincubation of target cells with certain plant lectins, such as phytohemagglutinin [7], suggesting that the IFN receptor system can be blocked by those lectins. On the other hand, gangliosides can bind to

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α- and β-type mouse and human IFNs and thereby block their antiviral [4, 6, 7, 13, 17], cell-growth inhibitory [4] and natural cytotoxicity boosting [10] activities, suggesting that the proposed cell membrane receptor for IFN contains a ganglioside-like component. Also, pretreatment of ganglioside-deficient mouse cells with exogenous gangliosides can cause an increase in sensitivity of these cells to IFN [17]. In an effort to further characterize the IFN receptor system, we studied the effect of glycolipids and glycophorin on IFN action.

EXPERIMENTAL

The human transformed cells, RSa [12], and GM258 cells were grown in monolayer cultures in Eagle's minimal essential medium (EMEM) supplemented with 5% fetal calf serum, 100 μg/ml of streptomycin and 100 units/ml of penicillin G, and incubated at 37°C in a 5% CO_2 atmosphere. Human IFN-β with specific activities of 6.6×10^5 units/mg protein and 7.5×10^7 units/mg protein were supplied respectively by Dr. A. Billiau (Rega Institute, University of Leuven, Belgium) and Dr. S. Kobayashi (Basic Research Laboratory, Toray Industries, Japan). Staphylococcal enterotoxin A-induced human IFN-γ (2×10^3 units/mg protein) and 45 K fraction of concanavalin A-induced human IFN-γ (7×10^6 units/mg protein) were supplied respectively by Dr. K. Cantell (Central Public Health Laboratory, Finland) and Dr. A. Billiau. Anti-human IFN-α and -β sera were supplied by Dr. A. Billiau. IFNs were treated with reagents for 30–60 min at 37°C, and the residual antiviral activity of IFN was measured by a virus yield reduction method [9, 12] and a cytopathic effect inhibition method [16]. None of the reagents, in the concentrations used in our experiments, showed any detectable effect on virus growth. GA1 was prepared by hydrolysing GM1 in formic acid at 100°C for 1 h. Sulfatide was obtained from bovine brain and glycophorin was obtained from human erythrocytes. Other glycolipids were prepared as described before [13]. *N*-Acetylneuraminic acid (NANA) was purchased from Sigma Chemical Co. (Saint Louis, MO, U.S.A.). The amount of gangliosides and glycophorin was calculated by determining the amount of NANA, and the amount of glycolipids was calculated by measuring the amount of glucose or galactose or both.

As shown in Fig. 1, concentrations of 30–80 μM of each of the gangliosides completely reversed the antiviral action of 100 units/ml of IFN-β. Glycophorin at a concentration of 120 μM also abolished the antiviral effect of 100 units/ml of IFN-β. The asialoganglioside, GA1, and globosides had moderate inhibitory effects, and concentrations of over 500 μM were required for complete inhibition. The glycolipids containing more than two sugars, trihexosyl ceramide and lactocyl ceramide, had weak inhibitory effects. Glucose ceramide which contains only one sugar had no effect on the antiviral activity of IFN-β, but sulfatide which contains sulfuric acid in addition to galactose had a moderate inhibitory effect. Neuramine lactose and NANA did not inhibit IFN-β. These data are summarized in Table 1 and suggest that the terminal acidic group, especially NANA, plays an important role in inactivation of IFN-β. This suggestion is supported

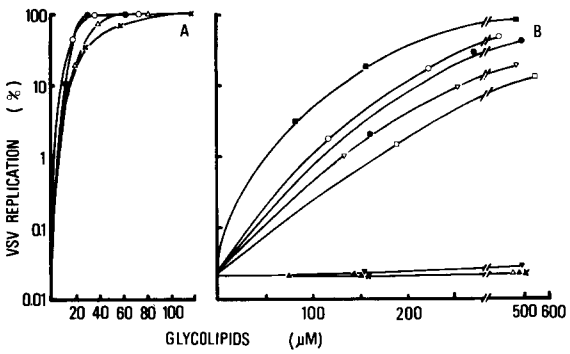

Fig. 1. Effects of glycolipids and glycophorin on the antiviral activity of human IFN-β. A) GM1 (○), GM2 (●), GM3 (△), glycophorin (×). B) GA1 (○), globoside (●), trihexosyl ceramide (□), lactosyl ceramide (■), galactosyl ceramide (△), glucosyl ceramide (▲), sulfatide (▼), neuramine lactose (▽), neuraminic acid (×).

by the fact that the glycoprotein, glycophorin, which contains NANA residues also had a strong inhibitory effect on the action of IFN-β. Glycolipids without NANA did retain a certain inhibitory effect when more than one sugar residue was present, also suggesting the view that the sugar chains are the active parts of the molecule.

TABLE 1

Glycolipids, glycoprotein and carbohydrates tested for inhibitory effects on human IFNs

Substrates tested	No of sugars per molecule	Acid	Inhibitory effect on	
			IFN-β	IFN-γ
Glycolipids				
Gangliosides				
GM1	4	NANA	+++	+
GM2	3	NANA	+++	+++
GM3	2	NANA	+++	++
Others				
GA1	4	-	+	-
Globoside	4	-	+	-
Trihexosyl ceramide	3	-	+	-
Lactosyl ceramide	2	-	±	NT[a]
Glucosyl ceramide	1	-	-	NT
Sulfatide	1	Sulfuric acid	+	NT
Glycoprotein				
Glycophorin	2[b]	2 × NANA[b]	+++	+
Carbohydrates				
Neuramine-lactose	2	NANA	-	NT
NANA	0	NANA	-	NT

[a] NT = not tested.
[b] Major component.

It has been suggested that binding of IFN-α and/or -β to cells might occur through gangliosides or a ganglioside-containing receptor. Since it has also been suggested that the cellular receptor site for IFN-γ may be different from that of IFN-α and/or -β [8], it was of interest to test the effect of gangliosides and glycophorin on the antiviral activity of IFN-γ. The results of these experiments are shown in Fig. 2. At 200 μM, GM2 almost completely inhibited the antiviral activity of 100 units/ml of IFN-γ, but GM1 and GM3 showed only moderate inhibitory effects. Glycophorin had only a weak inhibitory effect. GA1, globoside and trihexosyl ceramide did not significantly inhibit the activity of the IFN-γ preparations. It has been shown that mitogen-induced human IFN-γ preparations contain a component which is neutralized by anti-IFN-β serum [16]. Therefore, the inhibition seen with GM2 may at least partially result from the presence of IFN-β. In order to eliminate this possibility, a more purified preparation was used to do a confirmatory experiment. In particular, a 45,000 mol. wt. fraction consisting solely of IFN-γ was used to test the inhibitory effect of GM2. It can be seen in Table 2 that it was inhibited and that this inhibition was not affected by pretreatment of an IFN with antiserum that neutralized IFN-α and -β.

Ankel et al. [4] reported that mouse IFN-γ did not bind to ganglioside affinity column and that its activity was not blocked by gangliosides, but Aoyagi et al. [5] reported the partial inhibition of mouse IFN-γ activity by gangliosides. Our results showed that gangliosides could to a certain extent inhibit the antiviral activity of human IFN-γ. We cannot explain the difference between these results at this time. Pure interferons are needed to solve this discrepancy.

Recently, the number of specific binding sites for human IFN-α on a single cell has been calculated to be about 5000 for Daudi cell [8] and 1300 for FL cell (Yonehara, personal communication). Human fibroblasts contain large amounts of GM2 and GM3 [13]. Therefore, it seems improbable that these abundant molecules are solely responsible for specific binding and interaction of IFN with cells. Moreover, it has also been shown

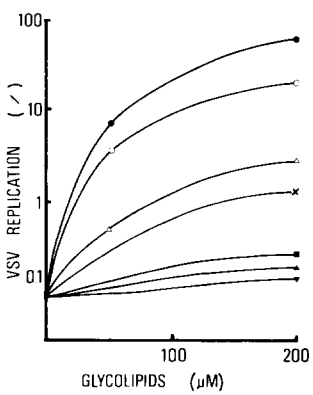

Fig. 2. Effects of glycolipids and glycophorin on the antiviral activity of human IFN-γ. GM1 (△), GM2 (●), GM3 (○), glycophorin (×), globoside (■), GA1 (▲), trihexosyl ceramide (▼).

TABLE 2

Effect of GM2 on the activity of partially purified IFN-γ

IFN preparation	Residual activity (log_{10} units/ml)	
	GM2 (200 μM) treatment	
	–	+
IFN-γ		
45 K fraction	2.2	1.0
45 K fraction pretreated with anti-IFN-α and -β	2.1	1.2
IFN-β	2.3	< 0.5

that IFN can bind to IFN-resistant cells [1, 2, 14]. Therefore, it remains possible that IFN binds aspecifically to gangliosides of the cell surface and that other as yet unknown components are subsequently responsible for allowing specific interaction with the cell. Accordingly, Grollman et al. [11] suggested that the IFN receptor is composed of two components: a binding site and an activator site. The results derived in the present study are compatible with this view.

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