Binding of interleukin 2 to gangliosides

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Exogenous gangliosides inhibit interleukin 2 (IL2)-dependent growth of a T cell line, AKIL-1.E8. IL2 activity is retained by columns of ganglioside covalently linked to poly(L-lysine)-agarose and is not eluted with ethylene glycol but is completely recovered by elution with 1% SDS. The ability of gangliosides to inhibit IL2 activity is directly related to the complexity of their carbohydrate portion, and related ceramide derivatives at similar concentrations do not inhibit IL2 activity. We conclude that IL2 bound to exogenous gangliosides is inactive and that the carbohydrate portion of the ganglioside is crucial to its interaction with IL2.

Ganglioside *Interleukin 2* *T cell growth factor* *Lymphokine* *T cell* *T lymphocyte*

1. INTRODUCTION

Gangliosides have been implicated in the mitogenic response of lymphocytes [1–4]. Mitogenic stimulation of T lymphocytes is a multi-step process involving production and action of IL2 [5]. Here we report that IL2-dependent T cell growth is inhibited by exogenous gangliosides and that inhibition is due to binding of IL2 to the glycolipid molecules. Our data suggest that T cell proliferation might be initiated by the interaction of IL2 with membrane gangliosides.

Abbreviations: IL2, interleukin 2; PBS, phosphate buffer; EG, ethylene glycol; Hepes, *N*-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; BSA, bovine serum albumin; globoside, GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer; GM_3, NeuNAcα2-3Galβ1-4Glc-Cer; GM_2, GalNAcβ1-4[NeuNAcα2-3]Galβ1-4Glc-Cer; GM_1, Galβ1-3GalNAcβ1-4[NeuNAcα2-3]-Galβ1-4Glc-Cer; GD1a, NeuNAcα2-3Galβ1-3GalNAcβ1-4[NeuNAcα2-3]Galβ1-4Glc-Cer; GD1b, Galβ1-3GalNAcβ1-4[NeuNAcα2-8 NeuNAcα2-3]Galβ1-4Glc-Cer; GT1b, NeuNAcα2-3Galβ1-3[NeuNAcα2-8 NeuNAcα2-3]GalNAcβ1-4Galβ1-4Glc-Cer

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2. MATERIALS AND METHODS

2.1. Materials

Human IL2 was obtained from pools of phytohemagglutinin stimulated lymphocytes from human blood and further purified (400-fold) by ammonium sulfate fractionation, DEAE–Sepharose column chromatography and gel filtration [6]. This preparation was free of lectin and had no interferon activity. After gel electrophoresis in 12% polyacrylamide with 0.1% SDS, we detect a prominent band of M_r 14 000 and a faint band migrating at the M_r of the BSA standard (66 000). The purified material was purchased from Boehringer Mannheim (Indianapolis, IN). Conditioned medium from Con A stimulated BALB/c mouse spleen cells was the source of mouse IL2 [7]. Rat IL2 (Rat T cell Polyclone) was from Collaborative Research (Lexington, MA).

Individual purified gangliosides were supplied by Seromed (Munich, FRG) and Supelco (Bellafonte, PA). Mixed gangliosides were supplied by Supelco, Sigma (St. Louis, MO) or ICN Pharmaceuticals (Cleveland, OH). Bovine brain cerebrosides were from Sigma and all other glycolipids were from Supelco.

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2.2. IL2 assay

IL2 activity was assayed by the growth response of an IL2-dependent cloned T cell line (AKIL-1.E8) [8]. AKIL cells do not grow in the absence of IL2, but respond to IL2 preparations of mouse, rat or human origin. AKIL cells are cultured in complete medium (Dulbecco's modified Eagle medium with added 10 mM Hepes, 14 μM folic acid, 2 mM L-glutamine, 0.3 mM arginine–HCl, 0.2 mM asparagine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 units/ml penicillin, 100 μg/ml streptomycin sulfate) supplemented with 5% fetal bovine serum and IL2.

For measurement of IL2 activity, 10^4 AKIL cells were cultured in 0.2 ml medium containing the dilution of IL2 for assay. For the last 24 h of a 3-day culture period, cells were supplied with 0.5 μCi [^{3}H]thymidine. Cells were collected on glass fiber filters and washed repeatedly with saline on a Bellco Cell Harvester. Dried filter discs were subjected to liquid scintillation counting. Thymidine uptake was a consistent measure of cell proliferation. By definition 1 unit of IL2 generates 50% of maximum uptake normalized to a standard preparation 100 units/ml (Rat T cell Polyclone).

2.3. Ganglioside inhibition

Gangliosides from bovine brain were used either in solution or immobilized on poly(L-lysine)-agarose by covalent attachment using the carbodiimide procedure [9]. Agarose beads were counted in a Nageotte hemacytometer.

Columns of agarose-ganglioside beads were prepared in plastic pipette tips and washed with 10 column volumes complete medium supplemented with 50 mg/ml BSA. A control column contained poly(L-lysine)-agarose beads to which sodium acetate had been covalently linked under conditions identical to those for coupling of gangliosides.

Sialic acid was determined by the resorcinol–HCl method [10]. Protein was determined by the fluorescamine [11] and Bradford methods [12] using a BSA standard.

3. RESULTS

The growth of IL2-dependent T cells (AKIL) is inhibited in a dose-dependent manner when the culture medium contains a mixture of bovine brain gangliosides in addition to either mouse or human IL2 (fig.1). The gangliosides are not toxic since preincubation of cells for 6 h with the same ganglioside mixture, followed by washing and subsequent culture in the presence of IL2, does not decrease thymidine uptake. Furthermore, gangliosides added after 42 or 66 h do not decrease thymidine uptake induced by IL2. The inhibitory effect of gangliosides persisted after the same ganglioside mixture was covalently attached to polylysine-agarose beads and then added to the cells (fig.1). In contrast, control polylysine-agarose beads are only slightly inhibitory. Since the agarose beads are many times bigger than the cells, it is not likely that the inhibitory action of gangliosides is due to their uptake into the cells. Inhibition of growth by agarose-ganglioside beads is not caused by release of free gangliosides into the solution as judged from the absence of IL2 inhibitory material in supernatants of these beads after incubation at 37°C for 4 days.

Since the major gangliosides in the ganglioside mixture used are monosialoganglioside GM1, the disialogangliosides GD1a and GD1b, and the

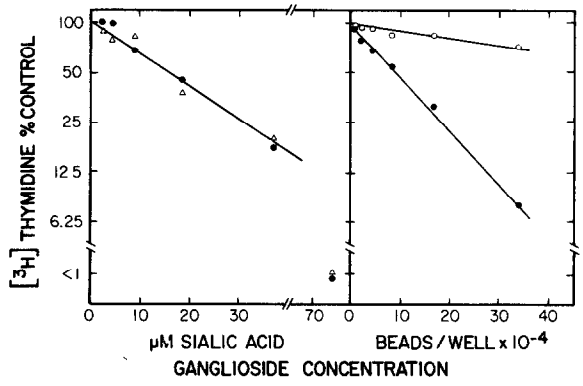

Fig.1. Inhibition of IL2 activity by bovine brain gangliosides. Left: human IL2 (2.2 units) (●) and mouse IL2 (1.4 units) (△) were preincubated with varying concentrations of bovine brain gangliosides prior to the addition of AKIL cells. Each point indicates the mean of triplicates. The ganglioside preparation used contained 0.74 μmol of sialic acid per mg. Right: the same bovine brain ganglioside mixture was covalently attached to poly(L-lysine)-agarose. There were 3×10^{-3} μmol of sialic acid per 10^4 beads. Rat IL2 (1.2 units) was preincubated with ganglioside-beads and then AKIL cells were added and the standard assay performed. (●) Ganglioside beads, (○) underivatized beads.

trisialoganglioside GT1b [13], we investigated their effects individually. All 4 gangliosides inhibit human IL2 activity (fig.2), yielding 50% inhibition of thymidine uptake at concentrations from 5 to 25 μM. All 4 gangliosides inhibit IL2 activity of rat or mouse origin in the same range (not shown).

If a series of individual purified gangliosides is compared in the ability to inhibit human IL2 activity, the molar concentration that produces 50% reduction in thymidine uptake decreases as the complexity of the carbohydrate portion of the molecule increases (e.g., GM3, 60 μM; GM2, 20 μM; GM1, 13 μM; GD1a, 8 μM). Similar concentrations of related ceramide derivatives with less carbohydrate complexity (bovine brain cerebrosides, Gaucher's cerebroside, lactosyl ceramide, ceramide trihexoside and globoside) do not inhibit either human or rat IL2.

To demonstrate that the inhibition of growth is due to direct interaction between IL2 and gangliosides, IL2 solutions were passed through columns containing agarose-ganglioside beads. These columns bound growth promoting activity contained in IL2 preparations of rat and human origin (table 1). The activity of IL2 added to eluates of ganglioside columns that have been washed only with complete medium plus BSA is the same as IL2 activity measured in the presence of complete medium plus BSA that has not passed through the column. Therefore, the failure to observe growth stimulation by column eluates is not due to the presence of gangliosides that have been released

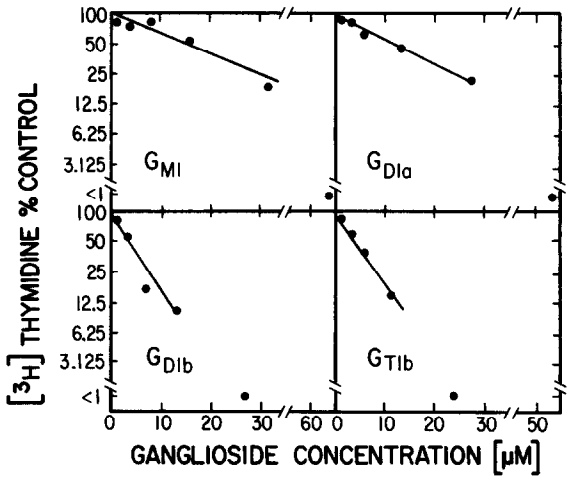

Fig.2. Inhibition of IL2 activity by individual gangliosides. Individual gangliosides of varying concentrations were preincubated with 2.2 units human IL2 prior to the addition of AKIL cells. Each point represents the mean of triplicates.

Table 1

Binding of IL2 to ganglioside beads

Column derivative	Column volume (ml)	IL2 source	Units applied	Units not retained (% of applied)	Units eluted (% of applied)	Second eluant (%)
Ganglioside	0.5	rat	26	ND		
Ganglioside	0.4	rat	80	ND	95	1% SDS[a]
Ganglioside	0.5	rat	120	8		
Ganglioside	0.5	human	11	ND		
Ganglioside	0.2	human	5	ND	5	20% EG[b]
Acetate	0.5	human	11	73		

[a] Column 2 was further eluted with 4 column volumes of complete medium supplemented with 1% SDS. The eluate was diluted and assayed for IL2

[b] Column 5 was prepared with PBS (pH 7.4) supplemented with 50 μg/ml BSA. After IL2 was applied and the column washed with PBS, the column was eluted with 4 column volumes of 50% ethylene glycol in PBS. The eluate was dialyzed against PBS and then assayed for IL2. Control IL2 that had been diluted 1:1 with 50% EG in PBS and then dialyzed with the column eluates retained full activity. ND, not detectable

Columns were prepared as described in section 2. IL2 was applied to the column and the material that passed through the column was collected along with a wash of 2 column volumes of complete medium plus 50 μg/ml BSA. The pooled eluate was assayed for IL2 to determine the units not retained by the column

from the agarose beads and block IL2 activity that itself has not been retained by the column. In contrast, more than 70% of the IL2 activity applied is not retained by a column of acetate-agarose beads. The interaction between IL2 and ganglioside-agarose beads is not reversed by treatment with ethylene glycol; but all the activity is eluted from the column under conditions where the protein was denatured with 1% SDS (table 1).

The ability of the ganglioside-bead columns to retain IL2 activity suggests that IL2 binds to gangliosides. The possibility that the IL2 binds to protein in the ganglioside preparations is unlikely. The ganglioside preparations contain small amounts of protein (1–4%). However, treatment of gangliosides with proteases (0.3 mg/ml trypsin or 0.25 mg/ml pronase) for 24 h prior to use fails to reduce their inhibition of human or rat IL2 activity. Ganglioside free cultures to which IL2 was added after inactivation of the proteases demonstrated normal growth. In addition, heating gangliosides (100°C for 15 min) does not reduce their inhibitory effect.

4. DISCUSSION

These results explain the ability of gangliosides to inhibit the mitogenic response of lymphocytes to IL2. Gangliosides bind IL2 and IL2 associated with gangliosides in solution cannot support T cell growth. The data agree with other reports that blastogenesis is inhibited when exogenous gangliosides are added to mitogen or lymphokine stimulated lymphocyte cultures [1–4].

One interpretation of this phenomenon is that the IL2 ganglioside binding which occurs in solution is representative of a similar interaction which normally occurs at the cell membrane. Others have shown that when chemically substituted gangliosides are incorporated into thymocyte cell membranes, cross-linking of the modified gangliosides with multivalent ligands specific for the ganglioside substituents leads to increased DNA synthesis [14,15]. The cross-linking of cell membrane gangliosides by multivalent ligands could stimulate DNA synthesis either directly, by mimicking the growth effect of IL2, or indirectly by initiating IL2 synthesis. The observations presented here suggest that membrane gangliosides or closely related structures interact with IL2 resulting in lymphocyte stimulation and further suggest that productive interaction of IL2 with target cells involves membrane-bound gangliosides or closely related structures.

Recently anti-Tac, a monoclonal antibody directed against a T lymphocyte surface antigen, has been shown to block IL2 activity and prevent binding of radiolabelled IL2 to a human cell line [16]. The highly glycosylated protein that binds anti-Tac has been proposed as a receptor for IL2 [17,18]. However, the data do not rule out the possibility that anti-Tac can also bind glycolipids or that glycolipids are intimately associated with the protein immunoprecipitated by anti-Tac. Antibodies that recognize both protein and glycolipid molecules have been described elsewhere [19] and the co-purification of protein and lipid is well known [20,21]. Our data suggest that protein is not responsible for the interaction of IL2 with gangliosides since rigorous heat or protease treatment of ganglioside preparations does not reduce IL2 inhibition. However, it remains possible that in the cell both glycoprotein and glycolipid act co-operatively in binding IL2 or that they bind IL2 with different affinities.

The specificity of the IL2-ganglioside interaction is indicated by the fact that other glycolipids structurally related to gangliosides do not inhibit IL2 activity. Furthermore, the ability of gangliosides to inhibit IL2 activity is directly related to the complexity of their carbohydrate moiety. This indicates that the carbohydrate portion of gangliosides is crucial to their interaction with IL2 and that the IL2–ganglioside interaction is more than a simple hydrophobic interaction. This conclusion is further supported by the failure of ethylene glycol to reverse the IL2–ganglioside interaction. Also, *N*-acetylglucosamine, a monosaccharide present in some gangliosides, has been shown to inhibit IL2 activity on rat thymocytes [22]. Thus, the binding of IL2 to gangliosides is primarily carbohydrate specific although some hydrophobic association may also occur.

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