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Article *in* Transplantation Proceedings · July 1994

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Immunomodulatory Effects of Glycosphingolipids on Lymphoproliferation and IL-2 Production in Rodents

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EFFECTIVE management and thorough understanding of the mechanisms involved in immune regulation remain the major barriers to clinical transplantation. Therefore, the search for novel immunosuppressive agents is necessary and justified. A number of experiments have reported that glycosphingolipids (GSLs) or their modified catabolites modulate transmembrane signal transduction at the cell surface by influencing protein kinases. On the other hand, the interaction between GSLs at the cell surface appears to modulate cell signaling, cell recognition, and adhesion to endothelial cells.[1–3] These properties of GSLs may be useful in the field of organ or cell transplantation.

In a previous study using GSLs extracted from *Leishmania amazonensis* amastigotes, inhibition of murine lymphocytes could be demonstrated in both concanavalin A (ConA) or lipopolysaccharide (LPS)-induced [^{3}H] thymidine uptake of normal and immunized BALB/c lymph node cells. Total GSLs also suppressed the two-way mixed lymphocyte reactions (MLR) of BALB/c × C57BL/6 cells and the antigen-specific response of immunized mouse cells.[4]

In this study we examined the effect of GSLs extracted from various Lewis rat (LEW) tissues on cell proliferation and function of spleen lymphocytes from either Lewis rats or BALB/c mice.

MATERIALS AND METHODS

Animals

Two hundred to 250 g adult male Lewis rats (n = 12) and 8-week-old male BALB/c mice were used. At the time of the experiment, animals were killed and the tissues harvested aseptically. Testis, mesenteric lymph node (MLN), spleen, pancreas, kidney, liver, brain, and eyes were excised from Lewis rats for GSL extraction. Spleens were collected from BALB/c mice and Lewis rats for cellular assays.

GSL Preparation

The quantitative isolation of total GSLs from brain, eye, testis, liver, pancreas, spleen, kidney, and MLN was done by acetylation of total lipids with pyridine and acetic anhydride. Separation of acetylated GSLs from non-GSL components was performed on a magnesia-silica gel column, deacetylation of GSLs was carried out in sodium methoxide 0.5%, and the GSL preparation was brought to neutral pH using Dewex at 50 $^+$H.[5] The isolated neutral GSLs were quantified by the phenol-sulfuric acid reaction.[6]

Proliferation Assays

Spleen cell suspensions were obtained by homogenization in RPMI 1640 with a loose tissue grinder. After washing, the cells were suspended in RPMI 1640 supplemented with 0.02 mmol/L sodium bicarbonate, 10 mmol/L HEPES, 3% fetal calf serum (FCS), 100 U/mL penicillin, 100 mg/mL streptomycin sulphate, 216 mg/mL L-glutamine, and 5×10^{-5} mmol/L b-mercaptoethanol. Cell viability was measured by trypan blue exclusion. Cells were plated in triplicate using 3×10^5 cells/well (BALB/c or LEW spleen mononuclear cells) in 96-well plates adding increasing amounts of GSLs (0.1, 0.25, 0.5, 1.0, 2.0 mg/well) and a fixed concentration of ConA (2.5 mg/mL) at the beginning of culture for GSLs from each organ individually. The culture plates were incubated at 37°C in 5% CO_2 for 48 hours. Cells were pulsed with 1 mCi of [^{3}H] thymidine/well 18 hours prior to harvesting on glass fiber filter paper for counting in a b-counter.

IL-2 Assay

IL-2 was assayed using supernatants of spleen lymphocyte cultures, stimulated with mitogen (ConA) in the presence of GSLs from liver and testis (at varied concentrations) on CTLL cell line (10^4/well), and incubated at 37°C in 5% CO_2 for 24 hours. Cells were pulsed with 0.5 mCi of [^{3}H] thymidine/well 6 hours prior to harvest on glass fiber filter paper and counting in a b-counter. Anti-IL-2 monoclonal antibody was used to ensure monospecificity for the lymphokine.

RESULTS

Data pooled from a minimum of four animals per group are shown in Tables 1 and 2. The inhibitory effect of neutral GSLs on lymphoproliferation suggests a dose-dependent pattern for both BALB/c and LEW cells. As shown in Table 1, inhibitory percentage was greater than 80% when lymphocytes from rat or mice were cultured in the presence of GSLs extracted from testis (0.5 to 2.0 mg/well) and others (data not shown). In contrast, GSLs from liver/brain required 1 mg/well to reach the equivalent inhibition level observed at mice lymphoproliferation. In addition, rat lymphocytes showed a lesser inhibition rate compared with the mice cells when GSLs from liver/brain were added. However, similar inhibition rate was observed for GSLs from testis/others. In the IL-2 assay, lymphocyte supernatants treated with GSLs from liver or testis showed a pattern of dose-dependent IL-2 inhibition that was in

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Supported by the CNPq, FAPESP, and FINEP of Brazil.

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0041-1345/94/$3.00/+0

Table 1. Rate of Inhibition (% ± SD) by [^{3}H] Thymidine Incorporation at 48 H in ConA-Stimulated Spleen Mononuclear Cells (LEW/BALB/c) in the Presence of Increasing Doses of GSLs Obtained From Varied LEW Organs

GSL Source	Spleen Lymphocytes	GSLs (μg/well) 0.1	0.25	0.5	1.0	2.0
Liver	BALB/c	6.4	42.9	65.5	81.6	95.7
Brain	BALB/c	4.5	30.5	60.2	75.6	92.0
Liver	LEW	nd	nd	9.2	36.1	35.4
Brain	LEW	nd	nd	6.5	22.8	30.7
Testis	BALB/c	66.5	88.6	94.7	95.5	96.2
Testis	LEW	nd	nd	84.9	93.6	94.7

Abbreviation: nd, not determined.

agreement with the lymphoproliferative inhibition. The rate of inhibition were calculated as described elsewhere.[4]

DISCUSSION

Included in the function of lymphocytes mediating cell rejection is the ability to proliferate and produce cytotoxic "killer" cells after alloantigen activation and to produce a number of lymphokine mediators that contribute to ongoing immune processes. We have thus investigated whether proliferation and IL-2 production are altered as a result of GSL treatment in the presence of ConA stimulation at the beginning of cell culture. Data shown above (Tables 1 and 2) suggest that in general there were significant changes in proliferation and IL-2 activity in a dose-dependent manner, GSL source, and in a nonspecific way for both LEW and BALB/c. In order to consider GSL application in cell or organ transplantation further studies are needed to elucidate the role of neutral GSLs and their purified components on immune induction. Despite the fact that the mechanism of action of GSLs is still under investigation, the results presented in this study are encouraging to further test GSLs within experimental animal transplantation models.

Table 2. Rate of IL-2 Activity Inhibition (% ± SD) by [^{3}H] Thymidine Incorporation at 24 H Culture Using CTLL Cell Line Cultured With Supernatants of ConA-Stimulated Spleen Mononuclear Cells (BALB/c) in the Presence of Increasing Doses of GSLs Obtained From Testis and Liver (LEW)

GSL Source	GSLs (μg/well) 0.1	0.25	0.5	1.0	2.0
Liver	nd	nd	10.2	30.2	85.3
Testis	50.2	69	90.4	nd	nd

Abbreviation: nd, not determined.

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