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Inhibition of Human Peripheral Blood Mononuclear Cell Proliferative Response by Glycosphingolipids from Metacestodes of *Echinococcus multilocularis*

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The effect on human peripheral blood mononuclear cells (PBMCs) of neutral glycosphingolipids extracted from metacestodes of the parasite *Echinococcus multilocularis* was investigated. Neutral glycosphingolipids inhibited [^{3}H]thymidine uptake by human PBMCs upon stimulation by mitogens such as phytohemagglutinin A and pokeweed mitogen or by allogeneic Burkitt B cells. This effect was dose dependent and was related to a decrease in interleukin 2 (IL-2) synthesis, the expression of IL-2 receptors (CD25) being unmodified. Addition of exogenous recombinant IL-2 restored the cell proliferation. Partial inhibition of immunoglobulin G (IgG), IgA, and IgM synthesis was observed in the supernatant of cell culture in association with the inhibitory effect. Identification of active subfractions contained in the neutral glycosphingolipid fraction was also studied in relation to cell viability. The free ceramide fraction had an inhibitory effect, in part related to cell lysis, particularly at high concentration, while the monogalactosylceramides had a paradoxical effect: as an activator at low concentrations and as an inhibitor at high concentrations, with limited cell survival. The immunogenic neutral glycosphingolipids containing at least two carbohydrate residues, all having a structure based on Galβ1→6Gal, were inhibitors of PBMC proliferation and showed good cell survival. These results suggest that parasite neutral glycosphingolipids may play an immunologically relevant role in alveolar hydatid disease.

Sphingolipids and, among them, glycosphingolipids as components of the surface membranes of all mammalian cells are thought to play a role in cell-cell and cell-matrix interactions that control growth and differentiation (13, 21). Similarly, these molecules may be expected to be involved in host-parasite interactions, such as species-related infestation and stage development or the choice of target organs that parasites preferentially invade. Moreover, glycosphingolipids have been shown to be immunogenic (19), and parasite glycosphingolipids, like other glycoconjugates (8), can stimulate the host immune response. Patients with *Trypanosoma*, *Leishmania* (1), or *Schistosoma* (38) infections were shown to express a humoral response with antibodies directed against parasite glycolipids. However, the effect of purified glycolipids in vitro on the host immune system was not established, except, to our knowledge, in mice for *Leishmania amazonensis* (9, 35).

Therefore, we investigated the effect on human peripheral blood mononuclear cells (PBMCs) of glycolipids of a cestode parasite, *Echinococcus multilocularis*. This parasite is responsible for alveolar hydatid disease, a severe human disease that can be fatal in the absence of efficient treatment. Metacestodes, a parasite stage found in humans, especially in the liver, have a complex pattern of neutral glycosphingolipids (NGSLs) with galactosylceramide as the major fraction (26, 27). The other neutral glycolipids present uncommon structures based on that of Galβ1-6Gal (28), which are expressed in other cestodes (4, 15, 24) as well as in a mollusk (20). In addition, the ceramide part of these glycosphingolipids is unusual, with sphinganine, a C_{18} saturated base, as a major base, and with fatty acid chain lengths ranging from 16 to 30 carbon atoms. Moreover, neutral glycolipids with at least two carbohydrate residues were able to induce an antibody response in alveolar hydatid disease human sera (31). In the present study, we have evaluated the potency of such parasite glycolipids in the modulation of human PBMC functions. The different compounds contained in the neutral glycolipid fraction were also tested separately to define what compound was active.

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MATERIALS AND METHODS

Animals. Metacestodes of *E. multilocularis* were taken from stock infection in Mongolian gerbils (*Meriones unguiculatus*) and were grafted intraperitoneally into 3-month-old gerbils. Ninety days postinfection, gerbils were killed by cervical dislocation, and metacestodes (which appeared as cysts) were collected, cleared from host tissue, and washed several times in 0.9% sodium chloride (30).

NGSL purification. The metacestodes were submitted to lipid extraction (30). The total lipid extract was acetylated and fractionated by Florisil column chromatography (33). After deacetylation, neutral glycolipids were separated from acid glycolipids on DEAE-Sephadex (A-25; acetate form) (36). They were then tested by thin-layer chromatography on precoated Silica gel 60 high-performance thin-layer chromatography plates with glass support (Merck; Darmstadt, Germany). NGSLs were separated in chloroform-methanol-water (60:35:8) and visualized as described previously with a stain specific for carbohydrates (26). As a control, neutral glycolipids from human erythrocytes (RBCs) were isolated by the same methods; however, they are structurally different from neutral glycolipids from *E. multilocularis* (Fig. 1, lane A).

The different constituents of the metacestode neutral glycolipid preparation were separated by preparative thin-layer chromatography in galactosylceramides and complex neutral glycolipids containing at least two carbohydrate residues. After localization by spraying the plate with water, the different fractions were scraped from the plate and eluted by sonication twice in chloroform-methanol (2:1) and then twice in chloroform-methanol-water (60:35:8) (17).

Control galactosylceramide from bovine brain (mixture of 50 to 60% α-hydroxylated fatty acids and 40 to 50% nonhydroxylated fatty acids) was purchased from Sigma, St. Louis, Mo. (reference C4905), and then was purified by preparative thin-layer chromatography as NGSLs.

Ceramide fraction. Lipid extract was applied to a silicic column of Bio-Sil A

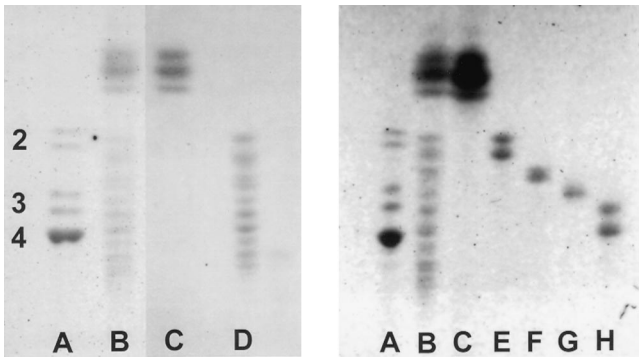

FIG. 1. High-performance thin-layer chromatography plates of glycolipids isolated from *E. multilocularis* metacestodes. (Left) Lanes: A, standard neutral glycolipids from human RBCs with the number of glycosidic residues indicated in the left margin; B, total NGSLs from *E. multilocularis* metacestodes; C, galactosylceramides from *E. multilocularis* metacestodes; D, other NGSLs from the parasite with at least two carbohydrate residues (NGSL≥2). (Right) Analysis of isolated NGSL≥2. Lanes A, B, and C are as described for the left panel. Lanes: E, fractions containing Galβ1→6Gal-Cer with major fatty acids $C_{26:0}$ and $C_{16:0}$; F, fractions containing Galβ1→6Gal-Cer with hydroxylated $C_{18:0}$ and Fucα1→3Galβ1→6Gal-Cer with $C_{16:0}$; G, fractions containing Fucα1→3Galβ1→6Gal-Cer with hydroxylated $C_{18:0}$; H, fractions containing Galβ1→6Galβ1→6Gal-Cer with $C_{26:0}$ or $C_{16:0}$.

(Bio-Rad Laboratories, Richmond, Calif.), as previously described (3). Neutral lipids and more polar neutral lipids were eluted with chloroform, and ceramides were eluted with chloroform-methanol (97:3 [vol/vol]). Ceramides were then purified by preparative thin-layer chromatography and collected as previously described (29). Control ceramide (reference C2137; Sigma) was from bovine brain and contained sphingosine associated with stearic and nervonic acids. The by-product controls sphinganine and palmitic acid were purchased from Sigma (references D6783 and P0500, respectively).

Chemical assays. The sphingoid base content of total glycolipids, neutral glycolipids, and sphingolipid fractions separated by thin-layer chromatography was assayed by the method of Naoi et al. (22). Sphingosine used as standard was purchased from Sigma (reference S6136).

Preparation and culture of PBMCs. Peripheral blood from healthy adults was collected on citrate buffer then defibrinated after addition of calcium chloride. Mononuclear cells were isolated by centrifugation on Lymphoprep (Nycomed AS, Oslo, Norway). The PBMC suspension contained at least 95% lymphocytes and was adjusted to 10^6 cells per ml in RPMI 1640 medium supplemented with 10% human AB serum heated at 56°C for 30 min and with antibiotics.

The fractions to be tested were diluted in absolute ethanol and then added to 96-well tissue culture microplate (Nunc) wells as 100-μl volumes. The microplates were left for 12 h at ambient temperature under sterile conditions in order to evaporate the solvent. Cells in culture medium were added to dried plates. In some experiments, fractions diluted in ethanol were added to culture medium as 20-μl volumes. Phytohemagglutinin (PHA) (Difco Laboratories, Detroit, Mich.) and pokeweed mitogen (PWM) (Difco) were used as mitogens at 40 μg/ml. In the mixed-lymphocyte culture, we used as a stimulator the B-cell line BL60, a group I Burkitt's lymphoma cell line bearing a surface phenotype characteristic of germinal center B blast cells. BL60 cells were treated for 1 h at 37°C with 0.25 μg of mitomycin (29805; Serva Heidelberg, Germany) per ml and then were extensively washed. BL60 cells were used at a ratio of one B cell per five lymphocytes. Recombinant interleukin 2 (rIL-2) (M 5273) from Pierce (Rockford, Ill.) was diluted in culture medium and added after the cells.

Cell proliferation was measured after addition of 0.5 μCi of [*methyl*-^{3}H]thymidine (CEA Saclay, France; specific activity, 1 Ci/mM) 4 to 12 h before the cells were harvested. Data are reported as mean disintegrations per minute of triplicate cultures. For cell viability measurements or cell surface and supernatant analyses, additional cultures were performed in the same conditions. Supernatants were frozen at −80°C, and the cells were used immediately.

Cells were numbered on a hemocytometer after dilution with 0.2% Trypan blue (vol/vol). Cell viability was assessed by cytofluorometry on cultured cells after addition of 5 μg of propidium iodide per ml.

Immunofluorescence assays. Phosphate-buffered saline (PBS) supplemented with 0.1% bovine serum albumin and 0.2% sodium azide was used as dilution medium for antibodies and as a washing solution for cells.

Fluorescein-conjugated CD25 (IL-2 receptor) monoclonal antibody was obtained from Becton Dickinson (Mountain View, Calif.); 20 μl of monoclonal antibody was added to 5×10^5 cell pellets for 30 min at 4°C. The cells were washed twice and then fixed in dilution medium with 1% formaldehyde (pH 7.4) and stored at 4°C.

Fluorescence was analyzed on a FACscan, and the data were analyzed with the LYSYS II program (Becton Dickinson) with a scatter gate for lymphocytes and blast cells that excluded monocytes, polymorphs, and dead cells.

ELISAs. Enzyme-linked immunosorbent assays (ELISAs) for determination of IL-2, immunoglobulin A (IgA), IgG, and IgM concentrations in cell culture supernatant have been described elsewhere (14, 37).

RESULTS

Inhibition of PBMC proliferation by parasite NGSLs. Ethanol solution containing various amounts of NGSLs from *E. multilocularis* metacestodes corresponding to the material in lane B (Fig. 1) was deposited on culture microplates. After solvent evaporation, the PBMC suspension was added. These PBMCs were then cultured at 37°C, and [^{3}H]thymidine incorporation was assayed by a pulse after 3 or 6 days of culture for the mitogenic or the allogeneic stimulations, respectively. The results in Table 1 indicated an absence of effect on spontaneous incorporation related to the addition of NGSL. On the other hand, the presence of parasite NGSLs induced a strong inhibitory effect on PBMCs activated with PHA or PWM. A similar effect was observed upon alloactivation with the B-cell line. Comparable results were obtained with direct addition of NGSLs in ethanol in cell culture; however, the solution needed to be more concentrated in order to introduce a maximum volume of 20 μl.

In a set of experiments, PBMCs were cultured for 1 h with NGSLs and mitogen. The cells were then washed and transferred to a new microplate and cultivated in fresh medium containing either NGSLs and PWM or PWM alone. Cells cultured with PWM alone were no longer inhibited (Fig. 2), whereas when cells were cultured 24 h before washing and transfer, the inhibition was conserved despite the absence of NGSLs in the culture medium.

As control, a NGSL fraction was isolated from human RBCs by the same methods used for *E. multilocularis* (Fig. 1, lane A).

TABLE 1. Effect of *E. multilocularis* NGSLs on spontaneous or induced proliferation of lymphocytes[a]

Medium	[^{3}H]thymidine incorporation (10^3 dpm ± SD)			
	Day 3		Day 6	
	Without NGSL	125 μM NGSL	Without NGSL	125 μM NGSL
RPMI	2.97 ± 0.52	3.85 ± 0.62		
+ PHA	121.6 ± 23.2	0.88 ± 0.13		
+ PWM	33.2 ± 4.11	0.88 ± 0.34		
RPMI			4.06 ± 1.27	4.53 ± 0.91
RPMI + mixed-lymphocyte culture			15.34 ± 1.28	1.26 ± 0.52

[a] The peaks of [^{3}H]thymidine incorporation were at day 6 for allogeneic stimulation and at day 3 for mitogenic stimulations. Proliferations at days 3 and 6 with solvent alone were (3.98 ± 0.84) × 10^3 dpm and (5.22 ± 1.32) × 10^3 dpm, respectively.

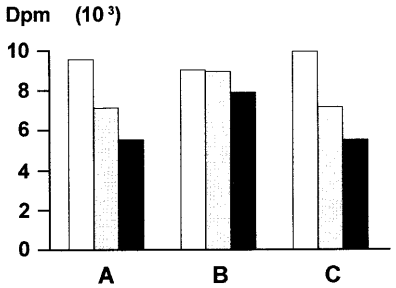

FIG. 2. Effect of limited incubations with NGSLs from *E. multilocularis*. PBMCs were incubated with 24 μM (solid bars) or 8 μM (shaded bars) NGSLs or culture medium (open bars). After 1 h of contact with NGSLs and PWM, cells were washed and transferred to a new microplate with medium containing NGSLs and PWM (A) or with medium containing PWM only (B). (C) Same protocol as group B but after 24 h of contact.

The dose-response effect of both preparations on PHA or PWM stimulation is presented in Fig. 3A and B. Both preparations progressively inhibited the proliferation of PBMCs with a 50% inhibition titer varying from 10 to 15 μM with NGSLs from RBCs and 40 to 50 μM with NGSLs from *E. multilocularis*. Whereas NGSLs from *E. multilocularis* did not modify the spontaneous [^{3}H]thymidine, NGSLs from human RBCs inhibited the background of proliferation in a dose-dependent manner.

PBMCs activated by PWM were cultured in the presence of various amounts of NGSLs. At day 2, CD25 expression on lymphocyte membrane was tested by immunofluorescence and IL-2 synthesis was assayed by ELISA of cell culture supernatants (Fig. 3C and D). IL-2 synthesis was inhibited by NGSLs of both origins, this effect being dose dependent, whereas CD25 expression remained stable. Addition of exogenous IL-2 allowed a total recovery of proliferation with NGSLs from *E. multilocularis* and only a partial recovery with those from human RBCs (Fig. 3B). Because PWM is a polyclonal B-cell activator in the presence of T lymphocytes, we measured by ELISA the production of Igs in cell supernatants at day 7 upon addition of various amounts of NGSLs from *E. multilocularis* (Fig. 3E). IgG, like IgA or IgM, was inhibited in a dose-dependent manner; however, IgM and IgA did not reach the level of the control value even with only 4 μM NGSL.

Comparative effect of NGSL and by-products on PWM activation. In order to analyze the components responsible for the effect on PBMC proliferation, different fractions contained in the NGSL extract (free ceramides; galactosylceramides, lane C in Fig. 1; complex NGSLs with at least two carbohydrate residues [NGSL≥2], lane D in Fig. 1) and different potential by-products were tested. [^{3}H]thymidine incorporation and CD25 expression were measured. The cell viability was checked for each fraction after 24 h of culture and after longer periods. The results are given in Table 2.

NGSL≥2 were all tested together because they were di-, tri-, and tetragalactosylceramides and fucolipids presenting a common structure with sphinganine as the major sphingoid base and with Galβ1→6Gal linkage (28). These complex glycosphingolipids inhibited the cell proliferation at higher concentrations (24 and 80 μM), in a dose-dependent manner, without a significant effect on cell viability or on CD25 expression. Further fractionation of NGSL≥2 in their basic constituents (Fig. 1, lanes E, F, G, and H) was performed, and all fractions were tested at 11 μM on PWM lymphocyte proliferation. The inhibition indices were 0.75 with fraction E, 0.53 with fraction F, 0.84 with fraction G, and 0.93 with fraction H, respectively. Fractions with a higher number of carbohydrate residues could not be tested because they represented amounts of material too small to be isolated with accuracy. NGSLs from erythrocytes used as a control had a high level of inhibition at 80 μM, but the inhibition was associated with a decrease in cell viability.

Parasite galactosylceramides contained one galactose residue associated with sphinganine (70.4% of the sphingoid base) and nonhydroxylated C_{16}-C_{30} fatty acids (79.9%) (27). They induced a paradoxical effect, with proliferation inhibition at a high concentration and activation at a low concentration. No significant effect on CD25 expression was observed, while cell viability decreased at day 1 with the higher concentration. Control galactosylceramide showed no effect on cell proliferation, with cell viability unmodified at day 5.

Parasite free ceramides contained 36.8% sphinganine and 63.2% sphingosine as sphingoid bases associated chiefly with $C_{24:1}$, $C_{16:0}$ fatty acids (29). They inhibited cell proliferation, particularly at 80 μM, with an absence of CD25 expression at this concentration and an almost complete absence of living cell recovery at day 5. Such an effect on CD25 and cell lysis was not observed in control ceramides that showed only a mild inhibition of PBMC proliferation.

Ceramide compounds, fatty acid and sphingoid bases, sphingosine, and sphinganine had very different effects. Sphingosine

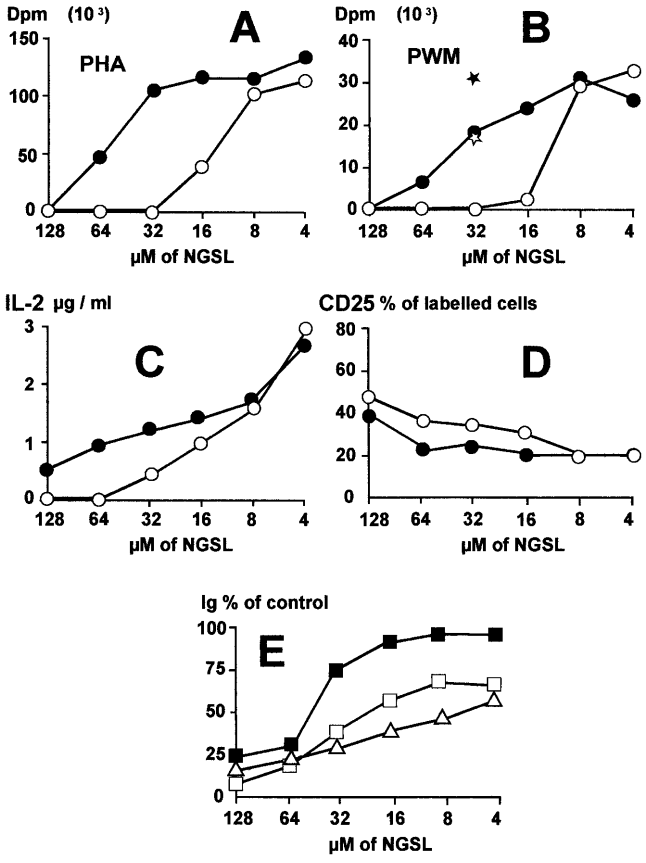

FIG. 3. Effect of total NGSLs from *E. multilocularis* metacestodes (●) or from human RBCs (○) on PBMC proliferation stimulated by PHA (A) or PWM (B). The effect of addition of 100 U of rIL-2 per ml is indicated by a solid star for *E. multilocularis* NGSLs and by an open star for human RBC NGSLs. Effect of NGSLs on IL-2 synthesis induced by PWM activation (C) and on CD25 expression (D). (E) Effect of NGSLs of *E. multilocularis* on the synthesis of IgG (■), IgA (△), and IgM (□) induced at day 7 by activation with PWM.

TABLE 2. Effect of NGSLs and related fractions on cell proliferation, CD25 expression, and cell viability with lymphocytes activated by PWM[a]

Sample	Proliferation at day 3			CD25 expression at day 2			Viability (cell no.) at:					
							Day 1			Day 5		
	80	24	8	80	24	8	80	24	8	80	24	8
E. multilocularis												
NGSL≥2	9,633	14,320	16,663	26.0	23.0	23.0	0.85	0.91	0.94	0.86	0.93	1.10
Galactosylceramide	15,622	33,847	57,019	23.5	27.1	28.8	0.48	0.68	0.69	0.66	0.64	0.79
Ceramide	6,509	13,799	12,758	7.36	27.1	23.4	0.72	0.84	0.89	0.04	0.67	0.68
RBCs, NGSL≥2	1,041	9,373	32,024	21.0	24.0	21.0	0.20	0.29	0.71	0.01	0.20	0.38
Other controls												
Galactosylceramide	27,077	24,213		30.6	29.9		0.83	0.82		0.60	0.68	0.64
Ceramide	11,456	12,758	23,432	21.0	24.0	21.0	0.78	0.85		0.62	1.05	
Sphingosine	260	25,255	45,823				0.01	0.83	0.99	0.00	0.65	0.75
Sphinganine	521	261	26,296				0.00	0.02	0.85	0.00	0.00	0.54
Palmitic acid	16,923	29,941	32,805				0.94	0.88	0.81	1.10	0.87	0.96

[a] Results of proliferation are expressed as disintegrations per minute of [^{3}H]thymidine incorporation; 26,036 dpm is counted in control cultures with solvent. CD25 results are expressed as percentage of cells labeled with anti-CD25 antibody; values for controls with solvent are 6% with unstimulated cells and 26% with PWM-stimulated cells. Cell viability is expressed as the index ratio of the corresponding response in the presence of solvent. These data represented one experiment over a set of three. 80, 24, and 8, 80, 24, and 8 μM shingoid base, with the exception of palmitic acid, which is expressed as 80, 24, and 8 μM fatty acid.

totally inhibited cell proliferation at 80 μM with a strong immediate cytolytic effect; the same effect was observed at 80 and 24 μM for sphinganine, while only a slight effect on cell proliferation was detected with palmitic acid.

DISCUSSION

This work represents the first in vitro study showing the effect of NGSLs from *E. multilocularis* metacestodes on peripheral mononucleated cells from healthy subjects. The addition of NGSLs from *E. multilocularis* directly to the culture of nonstimulated PBMCs, as shown in our controls, did not modify the spontaneous proliferation. NGSLs inhibited in a dose-dependent way the proliferation induced by a T mitogen such as PHA or a B mitogen requiring the presence of T cells such as PWM or alloantigenic cells. The inhibition was not the consequence of cell death, as indicated by viability measurements, but was due to a blockade of IL-2 synthesis. Indeed, CD25 expression was not impaired, and addition of exogenous rIL-2 restored the cell proliferation, indicating that the receptor was functional. The expression of newly expressed CD25 molecules associated with the blockade of IL-2 synthesis indicates that the first steps of lymphocyte activation are efficient. Ig determination at day 7 indicated a fall in Ig synthesis in PWM-activated PBMCs. This observation is not surprising, because an inhibition of T-cell proliferation was noticed at 72 h; however, we cannot exclude an additional effect of NGSLs directly on B cells. These results are in accordance with the results of Kizaki et al. (16) with spleen cells of mice. Kizaki et al. described a transitory decrease in proliferation of lymphocytes activated by concanavalin A in the presence of protoscolices of *E. multilocularis*. This decrease was associated with a fall in IL-2 synthesis, and addition of exogenous IL-2 allowed a recovery of cell proliferation. These results suggest a mechanism of inhibition comparable to the mechanism of action of NGSLs.

These data clearly indicated that NGSLs may play a role in nonspecific immunosuppression, as observed with protoscolices. In opposition to the absence of published data about the effect of NGSLs on lymphocytes, the inhibition induced by gangliosides, which are glycosphingolipids with sialic acid, is well documented (reviewed by Dyatlovitskaya and Bergelson [6]). Gangliosides bind rapidly to isolated lymphocyte membranes and bind more rapidly on intact cells. Whatever their origin, they were potent inhibitors of activated T and B lymphocytes. This inhibition is partially reverted by addition of exogenous IL-2 in excess, but the mechanism is now well understood and is different from the mechanism that we describe with NGSLs: IL-2 is normally synthesized, but gangliosides bind to IL-2 molecules, preventing their association with CD25. In our experiment, since cells responding to T cells still expressed functional CD25 in the presence of NGSLs but failed to produce IL-2, they displayed the typical phenotype of anergic cells described by Schwartz (34). The target of NGSLs can be the T cells or the accessory cells needed for complete T-cell activation, as suggested by the work of Rakha et al. (32). In this study, Rakha et al. showed, by using a proliferation test with normal spleen lymphocytes activated by concanavalin A, that peritoneal macrophages of BALB/c mice infested with *E. multilocularis* lose their accessory cell activity.

The effect of parasite glycosphingolipids on the proliferation of stimulated normal lymphocytes had been previously studied only with mice with NGSLs from the protozoon *L. amazonensis* (9). NGSLs inhibited the proliferation induced by concanavalin A, lipopolysaccharide, and alloantigens but there were no data allowing the mechanism of inhibition to be elucidated. However, the NGSL fraction was separated according to the number of osidic residues, and fractions with mono-, di-, tri-, and tetrahexosylceramides were more active than more complex fractions.

NGSLs from *E. multilocularis* represent a set of molecules made up with a various number of carbohydrate residues associated with a fatty acid and a sphingoid base, which is mainly sphinganine. That is why we used as controls several molecules corresponding to the components of NGSLs, such as fatty acid, sphingoid bases, ceramide, galactosylceramide, and molecules isolated from human RBCs. Among our controls, sphingoid bases induced a rapid disappearance of all cells in the culture with a lysis of PBMCs and RBCs. Under our conditions, the cell lysis observed was probably related to physical damage of cell membranes, since the effect was immediate and also concerned RBCs contaminating the PBMC preparation. Such a

cytolytic effect was also found at a high concentration in parasite ceramide fraction, contrary to that found with control ceramide. A few data were available on the cytolytic effect of natural sphingolipids. Often, C_2 ceramide analogs have been used, showing an 80% viability (2) and a cell proliferation inhibitory effect (5). Another ceramide analog inhibited mitogenesis stimulation by concanavalin A and PHA and inhibited the cell growth of the cytotoxic lymphoid line cells in the presence of IL-2 (7).

The cytolytic effect was to be considered, since inhibition of cell proliferation would appear to be related in part to the cell lysis and in part to a modification of the metabolic process. Such a cytolytic effect was less marked in parasite galactosylceramide than in ceramide alone and was almost absent in complex NGSL fractions. On the contrary, the inhibition observed with NGSLs from human RBCs was due to the addition of a cytolytic effect and a real inhibitory effect, because the addition of IL-2 restored a part of the proliferation. The inhibition observed on the background of proliferation in absence of stimulant may be related to the cytolytic potency of the fraction. The NGSLs from human RBCs were structurally different from parasite NGSLs, because the main sphingoid base is sphingosine instead of sphinganine and the first carbohydrate residue is glucose instead of galactose; moreover, as shown in Fig. 1, the most important component is globotetraosylceramide. Our results indicated that di-, tri-, and tetrahexosylceramides do not have the same activity on human PBMC proliferation after their origin; i.e., parasite or human RBCs.

The phenomenon of nonspecific inhibition of lymphocyte activation with NGSLs that we have described in vitro may have a counterpart in vivo. Several studies of alveolar echinococcosis already published help the hypothesis of a nonspecific in vivo inhibition by demonstrating that lymphocytes of infested patients or experimentally infested mice have a diminished proliferative response to nonspecific mitogens (12, 23). However, this counterpart in vivo was possible with NGSLs only if these molecules can be found in the bloodstream. NGSLs from human RBCs were probably hidden in mature erythrocytes (18) and therefore were inaccessible to lymphocytes. We have no direct evidence for NGSLs from the parasite; however, they are probably accessible in alveolar echinococcosis patient sera, as suggested by the detection of specific antibodies (31) and by the detection of immune complexes containing Galβ1→6Gal parasitic material and specific human IgG (25). Such complexes were detected with a sandwich ELISA with monoclonal antibody JA539 (10) specific for Galβ1→6Gal, which was used to coat the solid phase and a second antibody specific for human IgG. In a series of 24 patients with alveolar echinococcosis and 17 healthy subjects, the ELISA allowed the detection of immune complexes in 21 serum samples from patients and none of the control samples.

The findings of the present study suggest that glycosphingolipids may be an important class of molecules in parasite-host cell interaction. The blockade is associated in vivo with the production of specific antibodies, which until now has not been shown to exhibit a direct restricting role on the growth of the metacestode in humans (11). The conjunction of these two elements represents an important mechanism used by the parasite to survive and develop in the host.

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