Glycosphingolipids from *Sphingomonas paucimobilis* Induce Monokine Production in Human Mononuclear Cells

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Glycosphingolipids (GSL) isolated from the gram-negative lipopolysaccharide (LPS)-free bacterium *Sphingomonas paucimobilis* have remarkable structural similarities with LPS and its hydrophobic part, termed lipid A. Like LPS, but in contrast to the structurally related ceramides and cerebrosides, GSL contain an α -linked, negatively charged pyranosidic glycosyl component adjacent to the lipid portion and are capable of forming membranes. Because of these similarities, it was of interest to investigate whether these GSL are also able to induce monokine production in human mononuclear cells (MNC). Our results show that a GSL containing four sugar residues (GSL-4A) induced the release of tumor necrosis factor, interleukin-6, and interleukin-1 in MNC, whereas GSL-1, containing only one glycosyl residue, was inactive. A minimal concentration of 1 μ g of GSL-4A per ml was necessary to induce monokine production in MNC, whereas LPS was as active at a 10,000-fold-lower concentration (0.1 ng/ml). Both GSL-4A-induced monokine production and LPS-induced monokine production were reduced by the bactericidal/permeability-increasing protein and GSL-1. In contrast to LPS, GSL-4A-induced monokine release could be inhibited neither by an anti-CD14 monoclonal antibody nor by lipid A partial structures. We therefore conclude that at the receptor level, different mechanisms are involved in the LPS- and GSL-4A-induced monokine release.

Bacterial endotoxins are amphiphilic and complex constituents of the outer membrane of gram-negative bacteria (27). They are the most important toxins involved in human pathophysiological reactions such as fever, disseminated intravascular coagulation, and shock (26). Endotoxin effects are caused by lipid A, the endotoxic principle of lipopolysaccharide (LPS), which consists of two phosphorylated β 1,6-linked D-glucosamine residues acylated by up to six fatty acids in a specific manner (43). In an infected host, LPS (or lipid A) is able to induce the release of endogenous mediators, such as tumor necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1 (IL-1) (2, 6, 7, 26). However, in contrast to hexaacetylated *Escherichia coli* type lipid A, tetraacylated lipid A (synthetic compound 406 or natural precursor Ia) does not activate monocytes/macrophages but inhibits induction of monokines by LPS in a competitive way (19, 20, 22, 33, 34, 35). The prominent LPS-binding structure on monocytes/macrophages is the phosphoinositol-anchored membrane protein CD14 (14, 32, 39, 41). The binding of LPS to CD14 is necessary for the specific activation of human monocytes or murine macrophages (32, 39, 41). In the absence of a serum factor(s), this binding seems to be rather low, but it can be mediated by a soluble LPS-binding protein (LBP) or septins (25, 29, 31, 40). LBP forms a complex together with LPS and enhances binding to CD14 manyfold. Bactericidal/permeability-increasing protein (BPI) is another soluble LPS-binding protein. However, in contrast to LBP, BPI blocks the binding of LPS to monocytes (38) and thus the induction of monokines (8, 12).

In gram-negative bacteria, LPS is considered to be an obligatory constituent of the outer membrane which is responsible

for bacterial growth and survival (27). However, *Sphingomonas paucimobilis*, a strictly aerobic and nonfermentative gram-negative rod, was shown to have an outer cellular membrane completely devoid of LPS (18). Instead, two different glycosphingolipids (GSL) have been isolated from the cellular membrane, the tetraglycosylated GSL-4A and the monoglycosylated GSL-1 (18, 42), the structures of which are shown in Fig. 1. In these bacteria, GSL seem to be the most important amphiphilic constituents responsible for the proper architecture of the outer cell membrane. Different kinds of structural and physicochemical similarities between LPS/lipid A and GSL are striking: (i) they have similar ratios between negatively charged groups and fatty acids, (ii) computer modelling revealed that they are similar in architecture, in principle, and (iii) they are capable of forming membranes with similar physicochemical properties (37).

Biochemical studies on the stimulation of ceramide-activated protein kinase in myeloid cells revealed a close relationship of lipid A and ceramide (16). With regard to these postulated similarities, we investigated whether LPS and GSL have similar biological properties as well. We performed experiments in which the monokine release induced by LPS was compared with the monokine release induced by GSL. In particular, we investigated the mechanisms involved in the two processes to gain further insight into the structure-activity relationship between LPS and LPS-like structures with monocytes. We show that tetraglycosylated GSL-4A was able to induce the release of TNF, IL-6, and IL-1 by human mononuclear cells (MNC), whereas the monoglycosylated GSL-1 was inactive. Both BPI and GSL-1 were able to suppress LPS and GSL-4A activities, indicating some similarities between these two bacterial components. Despite these similarities between LPS and GSL, the GSL-4A-induced monokine release can be neither inhibited by an anti-CD14 monoclonal antibody (MAb) nor antagonized by a lipid A partial structure (compound 406),

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FIG. 1. (A) Chemical structures of the GSL of the LPS-free gram-negative bacterium *S. paucimobilis*, the monoglycosylated GSL-1 and the tetraglycosylated GSL-4A (modified by U. Zähringer and K. Kawahara).

indicating that different mechanisms are involved in the induction of GSL-4A- and LPS-mediated monokine release.

MATERIALS AND METHODS

LPS. LPS of *Salmonella friedland* was extracted by phenol-water and purified by the phenol-chloroform-petroleum ether method (11). This preparation contains less than 0.2% protein and is free of nucleic acids.

GSL-4A and GSL-1. GSL were extracted from *S. paucimobilis* IAM12576 with chloroform-methanol as described previously (18). The resulting lipids were successively eluted from a silica gel column by stepwise increases in methanol content. LPS was not detected in these preparations (as determined by gas-liquid chromatography-mass spectrometry by the lack of β -hydroxylated fatty acids and as also determined in the *Limulus* amoebocyte lysate test, kindly performed by D. Nitsche [Department of Surgery, University of Kiel, Kiel, Germany]).

Synthetic precursor 1a (compound 406). Synthetic tetraacyl lipid A precursor 1a (compound 406 or LA-14-PP) represents a partial structure of *E. coli* lipid A lacking dodecanoic and tetradecanoic acid. Compound 406 was synthesized as described elsewhere (15).

BPI. BPI is a 55-kDa basic protein found in granules of polymorphonuclear leukocytes. BPI is able to block LPS-induced TNF release in vivo and in vitro by binding to the lipid A domain (8). For our experiments, we used a recombinant amino-terminal fragment of BPI, rBPI₂₃ (12), which was kindly donated by P. Trown, Xoma Corporation, Berkeley, Calif.

Mabs. As an anti-CD14 Mab, we used MEM-18 (immunoglobulin G1) (3, 4) purified from murine ascites fluid by affinity chromatography on a protein A-Sepharose column (Pharmacia). MEM-18 was a kind gift from V. Horejsi, Prague, Czech Republic. Purified mouse immunoglobulin G1 (MOPC 21; Sigma GmbH, Deisenhofen, Germany) served as an isotype control.

Induction of TNF, IL-1, and IL-6 in human MNC. Human MNC from healthy donors were isolated by density gradient centrifugation (5) with Ficoll-Paque (Pharmacia, Uppsala, Sweden). After being washed three times, MNC (4×10^6 /ml) were stimulated at a volume of 200 μ l in U-form microtiter plates (Greiner, Nürtingen, Germany) in RPMI 1640 containing antibiotics and GSL or LPS. In costimulatory experiments, MNC were pretreated with compound 406 or GSL-1 for 1 h before GSL-4A or LPS was added. MNC were preincubated with

FIG. 2. GSL- and LPS-induced monokine production by MNC. MNC (4×10^6 /ml) were incubated with increasing concentrations of GSL-4A (10, 100, 1,000, and 10,000 ng/ml), GSL-1 (10, 100, 1,000, and 10,000 ng/ml), or LPS (0.01, 0.1, 1, and 10 ng/ml). After 16 h of culture, supernatants were harvested, and IL-1, TNF, and IL-6 activities were quantitated by bioassays. Results represent means $\pm$ standard deviations of triplicate cultures.

Mab MEM-18 at 4°C for 30 min before addition of the appropriate stimulus (GSL or LPS). In different costimulatory experiments, the stimuli were pretreated with rBPI₂₃ for 1 h before MNC were added. After 16 h of incubation at 37°C (5% CO₂), supernatants were harvested and immediately analyzed for cytokine activity or stored at -70°C.

Determination of TNF, IL-1, and IL-6 bioactivities. TNF, IL-1, and IL-6 activities were determined in bioassays as described elsewhere (10). In brief, TNF cytotoxic activity in culture supernatants was measured by using the TNF-sensitive L929 fibrosarcoma cell line. In the IL-1 bioassay, the proliferation-inducing activity of the culture supernatant from dermal fibroblasts isolated from human foreskin was determined (23). The IL-6 bioassay uses B9.3A4 cells (kindly provided by L. Aarden), which proliferate only in the presence of IL-6 (1). GSL-4A and GSL-1 preparations have been found to be without activity in all three bioassays.

RESULTS

GSL- and LPS-induced monokine production by MNC. Because of the biochemical and biophysical similarities between LPS and GSL, we investigated first whether GSL, like LPS, were able to induce monokines by MNC. Therefore, MNC (4×10^6 /ml) were incubated with increasing concentrations of GSL-4A or GSL-1 (10, 100, 1,000, and 10,000 ng/ml) or LPS (0.01, 0.1, 1, and 10 ng/ml). After incubation for 16 h, supernatants were harvested, and IL-1, TNF, and IL-6 activities were determined by bioassays (Fig. 2). The results show that GSL-4A, but not GSL-1, is able to induce IL-1, TNF, and IL-6 release by MNC in a dose-dependent manner. Comparison of the dose responses of GSL-4A and LPS indicates that a minimal concentration of 1 μ g of GSL-4A per ml is necessary for monokine induction, whereas LPS is active at a concentration

FIG. 3. Kinetics of TNF, IL-6, or IL-1 release by MNC stimulated with GSL-4A, GSL-1, or LPS. MNC (4×10^6 /ml) were incubated with GSL-4A (10 μ g/ml), GSL-1 (10 μ g/ml), or LPS (1 ng/ml) or without stimulus. After 2, 4, 8, 16, 20, 24, and 48 h of incubation, supernatants were harvested and monokine production was determined by bioassays. Results represent means $\pm$ standard deviations of triplicate cultures.

of 0.1 ng/ml and produces the highest stimulatory response at 1 ng/ml. Furthermore, Fig. 2 reveals that GSL-4A at 10 μ g/ml is comparable in biological activity to LPS at 1 ng/ml. Thus, the biological activity of LPS is almost 10,000-fold stronger than that of GSL-4A. Higher concentrations of GSL-4A were not tested, as only limited amounts of this compound were available. In contrast to LPS, the addition of fetal calf serum or human serum suppressed the monokine release induced by GSL-4A (data not given).

Kinetics of GSL- and LPS-induced monokine release. Next we compared the kinetics of monokine release induced by GSL and LPS. MNC (4×10^6 /ml) were treated with GSL-4A (10 μ g/ml), GSL-1 (10 μ g/ml), or LPS (1 ng/ml) at 37°C for 2, 4, 8, 16, 24, and 48 h. After harvesting of the supernatants, monokine release was determined in a bioassay. The results depicted in Fig. 3 show that GSL-4A-induced TNF release was first detectable after 8 h and reached its maximum after 16 h. In contrast, with LPS, MNC released biologically active TNF after only 4 h of stimulation, with the maximal release being attained after 8 h. The time course of IL-1 release determined in the same supernatant as TNF release was similar. As was the case for TNF, GSL-4A-induced IL-1 production reached its maximum after 16 h. IL-1 induced by LPS even increased after 20 h to a maximal level in the 48-h samples. In the same experiment, IL-6 release was determined. IL-6 release induced by GSL-4A was first detectable after 8 h and increased within time, but in contrast to the results for TNF and IL-1, maximal

TABLE 1. Comparison of GSL-induced monokine production by MNC or monocytes^a

Cells	Monokine	Monokine production (U/ml)		
		GSL-4A (10 μ g/ml)	GSL-1 (10 μ g/ml)	Control
MNC	TNF	44 $\pm$ 8	<1	<1
	IL-1	236 $\pm$ 2	<1	38 $\pm$ 5
	IL-6	74 $\pm$ 14	2 $\pm$ 1	2 $\pm$ 1
Monocytes	TNF	29 $\pm$ 5	<1	<1
	IL-1	343 $\pm$ 32	<1	<1
	IL-6	81 $\pm$ 12	<1	1 $\pm$ 1

^a MNC (4×10^6 /ml) or monocytes (10^6 /ml) of the same donor were stimulated with GSL-4A or GSL-1 or were not stimulated (control). After incubation for 16 h at 37°C, supernatants were harvested and monokine release was measured by bioassay. Results represent means $\pm$ standard deviations of triplicate cultures.

levels of IL-6 were reached at 20 h. Again, LPS-induced IL-6 was detectable after 4 h and reached maximal concentrations within 16 h of MNC incubation. GSL-1 was not capable of inducing monokines during the culture times under the conditions tested.

Comparison of GSL-induced monokine release by MNC and monocytes. After determining the stimulatory capacity of GSL-4A in MNC, we attempted to define more precisely the actual target cells. Purified human monocytes (10^6 /ml) or human MNC (4×10^6 /ml) of the same donor were incubated with GSL-4A (10 μ g/ml) or GSL-1 (10 μ g/ml) or without stimulus. After 16 h of incubation, IL-6, TNF, and IL-1 activities were determined in supernatants by bioassays (Table 1). The results obtained indicate that purified monocytes also release IL-6, TNF, and IL-1 after stimulation with GSL-4A. Again, it was confirmed that GSL-1 lacked monokine-inducing capacity. It appeared that GSL-4A-induced IL-6 and IL-1 secretion by monocytes was higher than that by MNC. In contrast, TNF release by monocytes was slightly lower than that by MNC.

Effect of an anti-CD14 MAb on GSL-4A- or LPS-induced monokine production. It is known that CD14 is involved in the recognition of LPS by MNC (13). Therefore, we investigated whether CD14 was also involved in GSL-4A-induced monokine release. MNC (4×10^6 /ml) were incubated with increasing concentrations of anti-CD14 MAb MEM-18 for 20 min at 4°C before addition of GSL-4A (10 μ g/ml) or LPS (1 ng/ml). After 16 h of incubation, supernatants were harvested, and IL-1 and IL-6 activities were quantitated in bioassays. The anti-CD14 MAb MEM-18 alone had no cytokine-inducing capacity (Fig. 4). The isotype control mouse immunoglobulin G1 (MOPC 21) reduced neither GSL-4A- nor LPS-induced monokine production (data not shown). Figure 4 shows that addition of the anti-CD14 MAb resulted in a complete inhibition of LPS-induced IL-6 and IL-1 release. In the case of GSL-4A, we found that the anti-CD14 MAb was not able to inhibit GSL-4A-induced monokine production but that the addition of the anti-CD14 MAb led to a clear enhancing effect of GSL-4A-induced monokine release, which was mainly detectable at low concentrations of antibody (1 to 9 μ g/ml).

Effect of lipid A partial structures on GSL-4A- or LPS-induced monokine release. We next examined whether lipid A partial structures, such as compound 406, would have an inhibitory effect on GSL-4A-induced IL-6 and IL-1 release. MNC (4×10^6 /ml) were preincubated with increasing amounts of compound 406 for 1 h at 37°C. Thereafter, the appropriate stimulus (GSL-4A at 10 μ g/ml or LPS at 1 ng/ml) was added. After 16 h of MNC incubation, supernatants were harvested, and monokine release was determined in a bioassay (Fig. 5). In

FIG. 4. Effect of an anti-CD14 MAb on GSL-4A- or LPS-induced monokine release. MNC (4×10^6 /ml) were preincubated with increasing concentrations of anti-CD14 MAb MEM-18 as indicated for 20 min at 4°C, and then GSL-4A (10 µg/ml) or LPS (1 ng/ml) was added. After 16 h of culture, supernatants were harvested, and IL-6 and IL-1 release was measured by bioassays. Results represent means $\pm$ standard deviations of triplicate cultures.

the case of LPS, the results obtained confirm previous findings (10, 36) and demonstrate that LPS-induced IL-6 and IL-1 release could be blocked in a dose-dependent manner by compound 406. Complete inhibition of cytokine production was obtained with 1,000 ng of compound 406 per ml. Even 100 ng

FIG. 5. Effect of the lipid A partial structure compound 406 on GSL-4A- or LPS-induced IL-1 and IL-6 release. MNC (4×10^6 /ml) were preincubated with increasing concentrations of compound 406 for 1 h at 37°C before GSL-4A (10 µg/ml) or LPS (1 ng/ml) was added. After 16 h of incubation, supernatants were harvested, and IL-6 and IL-1 activities were quantitated by bioassays. Results represent means $\pm$ standard deviations of triplicate cultures.

FIG. 6. Effect of rBPI₂₃ on GSL-4A- or LPS-induced IL-6 and IL-1 release. GSL-4A (10 µg/ml) or LPS (1 ng/ml) was preincubated for 1 h with increasing concentrations of rBPI₂₃ before MNC (4×10^6 /ml) were added. After 16 h of incubation, supernatants were harvested and IL-6 and IL-1 activities were determined by bioassays. Results represent means $\pm$ standard deviations of triplicate cultures.

of compound 406 per ml was able to reduce LPS-induced monokine release by more than half. As expected, compound 406 alone was not able to induce IL-6 and IL-1 release by MNC (data not shown). In contrast, compound 406, added to the cells 1 h before stimulation with GSL-4A, had no inhibitory effect on IL-6 and IL-1 production at the concentrations tested.

Effect of rBPI₂₃ on GSL-4A-induced monokine release. rBPI₂₃ is known to block LPS-induced monokine release in vivo and in vitro (12) by binding to the lipid A domain. Because of the structural similarities between LPS and GSL, it was of interest to test whether rBPI₂₃ would also inhibit GSL-4A-induced monokine release. GSL-4A (10 µg/ml) or LPS (1 ng/ml) was preincubated with rBPI₂₃ (100, 1,000, and 10,000 ng/ml) for 1 h at 37°C before addition to MNC (4×10^6 /ml). After 16 h, IL-1 and IL-6 activities were quantitated in bioassays (Fig. 6). The results obtained show that rBPI₂₃ at 10 µg/ml blocks GSL-4A-induced IL-1 release completely and IL-6 production by two-thirds. The results for LPS are in line with earlier findings showing that LPS-induced IL-1 as well as IL-6 release can be completely inhibited by rBPI₂₃ (10 µg/ml). rBPI₂₃ alone was not able to induce monokine release by MNC (data not shown).

Effect of GSL-1 on GSL-4A- or LPS-induced IL-1 and IL-6 production. To further characterize the mechanism of GSL-4A-induced monokine production, the effect of the inactive, monoglycosylated GSL-1 on GSL-4A- and LPS-induced IL-1 and IL-6 release was investigated. MNC (4×10^6 /ml) were preincubated with increasing concentrations of GSL-1 for 1 h at 37°C before the stimulus (GSL-4A at 10 µg/ml or LPS at 1 ng/ml) was added. After incubation for 16 h, IL-1 and IL-6 activities were assayed in the culture supernatants (Fig. 7). The results show that LPS-induced IL-1 release is significantly inhibited by GSL-1 at a concentration of 1 µg/ml and is completely suppressed by 50 µg/ml. LPS-induced IL-6 production

FIG. 7. Effect of monoglycosylated GSL-1 on GSL-4A- or LPS-induced IL-6 or IL-1 release. MNC (4×10^6 /ml) were preincubated with increasing concentrations of GSL-1 (1, 10, and 50 μ g/ml) for 1 h at 37°C before GSL-4A (10 μ g/ml) or LPS (1 ng/ml) was added. After 16 h of incubation, supernatants were harvested, and IL-6 and IL-1 activities were measured by bioassays. Results represent means $\pm$ standard deviations of triplicate cultures.

was not affected by 1 or 10 μ g of GSL-1 per ml. However, 50 μ g of GSL-1 per ml was able to inhibit LPS-induced IL-6 release almost completely. In the case of GSL-4A, 50 μ g of GSL-1 per ml reduced both IL-1 and IL-6 production by half.

To exclude the possibility that the inhibitory effects of GSL-1 on GSL-4A- or LPS-induced monokine release described above were due to toxic effects of GSL-1 on purified monocytes, the viability of purified monocytes after incubation with GSL-1 was analyzed. After 16 h of incubation, the viability of monocytes was not significantly affected by the concentrations of GSL-1 (up to 50 μ g/ml) tested (data not shown).

DISCUSSION

The aim of this study was to determine whether GSL of *S. paucimobilis* were capable of inducing cytokines in human MNC. The results of the study presented clearly show that the tetraglycosylated GSL-4A is able to induce TNF, IL-6, and IL-1 release by MNC, whereas the monoglycosylated GSL-1 is completely inactive. Furthermore, purified monocytes are able to produce IL-6, TNF, and IL-1 release following stimulation with GSL-4A but not with GSL-1.

The dose-response study of cytokine production showed that GSL-4A is biologically active only at a concentration 10,000-fold higher (10 μ g/ml) than that of LPS (less than 1 ng/ml). Different responses of the cells to GSL-4A or LPS were also found in kinetic experiments. Time course studies of cytokine release from GSL-4A- and LPS-stimulated MNC revealed that the maximal GSL-4A-induced monokine release was within 16 h of incubation. GSL-4A-induced TNF was first detectable after 8 h and reached the maximum within 16 h. IL-6 induced

by GSL-4A was also measurable after 8 h, whereas LPS-induced TNF, IL-6, and IL-1 could be detected after only 4 h. Taken together, the results show that the biological activity of GSL-4A was detectable at a concentration of 10 μ g/ml after 16 h of incubation.

As CD14 constitutes a prominent LPS-binding structure on monocytes/macrophages (32, 39, 41), we examined whether GSL-4A-induced activation of MNC is also CD14 dependent. Our results demonstrate that pretreatment of MNC with anti-CD14 MAb MEM-18 did not result in an inhibition of GSL-4A-induced monokine release. In contrast, the addition of anti-CD14 MAb MEM-18 led to a clear enhancement of monokine production. This phenomenon may be explained by cross-linking of cell-bound anti-CD14 with anti-CD14 MAb MEM-18, thus generating an intracellular signal. Such cross-linking of cell-bound anti-CD14 causes signal transduction, in this case cytoplasmic Ca^{2+} fluxes and oxidative burst in human monocytes, has been reported elsewhere (21, 24). However, the anti-CD14 MAb that we used in our experiments is not active itself in inducing monocytes, and therefore it seems more likely that the above-mentioned enhancement of monokine production is due to a synergistic effect.

To get further insight into the mechanism of GSL-4A-induced monokine release, we investigated the effect of compound 406 on the activation of MNC after stimulation with GSL-4A. Compound 406 was shown to be not an agonist (9) but an antagonist of LPS-induced monokine production (19, 22, 33, 34, 35). We also found that compound 406 has no effect on GSL-4A-induced IL-6 and IL-1 release, again suggesting that there are different mechanisms involved in the activation of MNC by GSL-4A and LPS.

An interesting finding is that rBPI₂₃, known to inhibit LPS bioactivity, is also capable of inhibiting GSL-4A-induced IL-1 and IL-6 release. Gazzano-Santoro et al. (12) demonstrated that rBPI₂₃ inhibits LPS-induced monokine release by binding to the lipid A domain. The binding site of rBPI₂₃ to GSL, however, is not known at present, and the interpretation of our results therefore requires further study.

Monoglycosylated GSL-1 lacked bioactivity and was furthermore considered an inhibitor of cytokine induction as reported for structurally similar gangliosides (44). We found that GSL-4A-induced IL-1 and IL-6 release was inhibited in a dose-dependent manner by GSL-1, resulting in a 50% inhibition at a concentration of 50 μ g of GSL-1 per ml. An inhibitory effect could also be observed for LPS-induced monokine release. In this case, GSL-1 was found to be a more potent antagonist, inhibiting almost completely the LPS-mediated response at a concentration of 50 μ g/ml. Viability tests (trypan blue exclusion) demonstrated that GSL-1 did not have toxic effects in the tested concentrations of monocytes. The inhibitory effect of GSL-1 might be explained by its physicochemical structure and/or by its mode of interaction with the responding cells. Amphiphilic molecules like GSL or LPS tend, depending on their hydrophobicity and concentration, to form supramolecular structures (30). If GSL-1 possesses a much lower state of order (higher fluidity) than GSL-4A or LPS, the micellar structure of GSL-1 might inhibit the aggregates formed by GSL-4A and LPS just by absorption. On the other hand, GSL-1 might also specifically interact with a cell-bound receptor protein responsible for the binding of GSL-4A or LPS.

Studies on ceramide stimulation of protein kinase in myeloid cells led Joseph et al. (16) to the conclusion that the lipophilic portion of the LPS, i.e., the lipid A part, can also express a kinase-stimulating activity such as that found for ceramides. The authors further tried to support this idea by using molecular modelling and found a structural similarity between cer-

amide and a region of lipid A. We also performed such molecular modelling studies based on the data of Kastowsky et al. (17) and also found similar physicochemical structures of glycosphingolipids and lipid A (37). Since ceramides and sphingolipids have structural similarities in their lipid parts, these data should stimulate further investigations on structure-function relationships in both systems, i.e., in myeloid cells (16) and human MNC. However, since little is known about the conformation and the physicochemical behavior of these amphiphilic molecules in aqueous solutions and binding structures for GSL have not been determined, additional studies are necessary to clarify the exact mechanism involved in the action of GSL.

In this study, we demonstrated for the first time that the tetraglycosylated GSL-4A, but not the monoglycosylated GSL-1, is able to induce monokines by MNC. Although the physicochemical behavior of GSL-4A is remarkably similar to that of LPS, the mechanism of GSL-4A-induced monokine release seems to be different from that of release induced by LPS. The differences in the bioactivities of GSL-4A and LPS may be due to different physicochemical behaviors of these amphiphilic components or to different activation pathways and receptors involved in the induction of monokines. Interestingly, GSL-4A is similar in structure to ceramide. Therefore, GSL-4A may stimulate a ceramide-activated protein kinase and, like LPS (16), may mimic the second-messenger function of ceramide. However, these properties of GSL-4A and LPS require further investigation. Nevertheless, our results provide additional information on the structure-activity relationships of LPS and LPS-like structures with MNC and thus contribute to answering one of the pivotal questions about the mechanism whereby MNC recognize LPS and generate transmembrane signals.

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