

## IMMUNOCHEMICAL DETERMINATION OF GANGLIOSIDE GM<sub>2</sub>, BY INHIBITION OF COMPLEMENT-DEPENDENT LIPOSOME LYSIS

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Immunochemical quantitative determination of a lipid antigen, ganglioside GM<sub>2</sub>, has been developed, based on the inhibition of the immune lysis of liposomes containing the antigen in their lipid bilayer. It has been shown that the full expression of the antigenicity of the competing lipid requires its dispersion in accessory lipids. The assay of inhibition of liposome lysis can be used also for the establishment of the antigenic similarity of structurally related lipid antigens.

### INTRODUCTION

The interaction of specific antibodies with lipid antigens has attracted much interest in recent years. In these systems, in contrast to most proteins, sugars or artificial antigens, the main difficulty in the analysis of the antigen-antibody reaction stems from the fact that lipids are insoluble in aqueous solutions which are optimal for antibody activity, and tend to form macromolecular aggregates of micellar or lamellar nature.

However, it has been shown that lipid antigens may be incorporated into the bilayer shells of liposomes and that the immune reaction may take place at their surface (reviewed by Niediek, 1975; Alving, 1976).

Kinsky and his group have systematically followed the interaction of various lipid or derivatized lipid antigens, including the Forssman antigen and DNP-bound phosphatidyl ethanolamine, with their respective antibodies, by the complement-mediated lysis of liposomes into which the relevant antigen has been incorporated. The extent of lysis has been thus determined by the release of entrapped markers such as glucose (Haxby et al., 1968; Kinsky et al., 1969) fluorescein isothiocyanate (Sato and Hara, 1972), spin label (Humphries and McConnell, 1974; Wei et al., 1975), enzymes (Kataoka et al., 1973), etc. We have recently proposed the use of a complex of the highly water-soluble fluorophore 1-aminonaphthalene-3,6,8-trisulfonate (ANTS) with the quencher dipyrindinium-*p*-xylene (DPX) for a continuous recording of the antibody-mediated complement lysis of liposomes (Smolarsky et al., 1977). The complex is entrapped in the inner aqueous volume of liposomes, into the membranes of which an antigen has been

incorporated. Upon addition of antibodies and complement, lysis occurs and the fluorophore-quencher complex is released and diluted in the external volume. Thus the quench is abolished and a fluorescent signal is recorded.

In the present study we have used this system for the immunochemical quantitative determination of ganglioside GM<sub>2</sub> by the specific inhibition of the immune lysis of liposomes.

## MATERIALS AND METHODS

### *Buffer systems*

Buffer A: 0.1 M Tris-HCl, pH 7.5 containing 0.025 M NaCl and 0.025 M KCl. Buffer B: the same as buffer A, but in addition contains  $3.5 \times 10^{-3}$  M MgCl<sub>2</sub> and  $1.5 \times 10^{-4}$  M CaCl<sub>2</sub>.

Lipids: Phosphatidyl choline (type IIIE, PC) was purchased from Sigma (U.S.A.), dicetylphosphate (DCP) from ICN K & K Laboratories (U.S.A.), cholesterol (Chol) from Merck (Germany) and DL- $\alpha$ -tocopherol ( $\alpha$  Toc) from Assia Chemical Laboratories (Israel). Ganglioside GM<sub>2</sub> was purified from Tay-Sachs brain following the general procedure of Svennerholm (1972). Globoside\* was extracted and purified from human stromata according to Yamakawa et al. (1960). Both the ganglioside and the globoside appeared as single bands in thin layer chromatography on Silica gel G-pre-coated plates in several solvent systems (Merck, Germany). Sphingomyelin was bought from Sigma (U.S.A.), galactocerebroside and sulfatide were a gift from Dr. Y. London and appeared almost as single spots on thin layer chromatography in several solvent systems. Pure glucosyl ceramide was a gift from Dr. Z. Ben-Gershon.

### *Complex between ANTS and DPX*

1-aminonaphthalene-3,6,8-trisulfonic acid was conjugated to dipyrindinium-*p*-xylene as described previously (Smolarsky et al., 1977).

### *Antisera*

Antisera to ganglioside GM<sub>2</sub> were raised in rabbits by 3 weekly injections of aqueous solution of GM<sub>2</sub> (5 mg each) emulsified in an equal volume of complete Freund's adjuvant (DIFCO). The animals were bled and sera prepared 10 days following the last injection. In parallel, rabbits were also immunized with a mixture containing 5 mg of BSA and 5 mg of GM<sub>2</sub>. Non-immune sera were prepared from rabbits immunized with non-relevant antigens in complete Freund's adjuvant. All sera were heat-inactivated (40 min at 56°C) and tested for the absence of complement activity before use.

\* Globoside: N-acetylgalactosaminyl<sup>1→3</sup> galactosyl<sup>1→4</sup> galactosyl<sup>1→4</sup> glucosyl<sup>1→1</sup> ceramide.

### *Complement*

Guinea pig complement was obtained from Difco (U.S.A.). The source for human complement was human serum which was prepared at 4°C and kept at -80°C in small aliquots until used.

### *Fluorescent liposomes*

Liposomes containing entrapped ANTS-DPX complex have been prepared essentially as described previously (Smolarsky et al., 1977). The lipid mixture (MIX A) consisted of PC : Chol : DCP :  $\alpha$ TOC in molar ratio of 2 : 1.5 : 0.04 : 0.2, to which the antigen was added in the organic solvent, prior to liposome formation.

The liposomes were separated from the unentrapped fluorophore by gel filtration through Sepharose 6B column (Pharmacia, Sweden).

The fluorescent liposomes, as well as those which did not contain fluorescent dye and were used for the specific inhibition of the lysis, were multilamellar and were prepared by vigorous mixing on a Vortex.

GM<sub>2</sub>-containing liposomes with entrapped ANTS-DPX complex have been prepared by dissolving 0.3 mg GM<sub>2</sub> and 2 mg of MIX A lipids in chloroform-methanol 1 : 1. Liposomes were then prepared as described in a previous publication (Smolarsky et al., 1977). Thus GM<sub>2</sub> concentration is 13% of the total lipids (on weight basis) and 10% of the PC, on molar basis. The liposome suspension, eluted from the Sepharose 6B column (0.5 ml), was further diluted  $\times 5$  with buffer A before use. An aliquot of 10  $\mu$ l of this liposome suspension containing 0.9 nmoles of GM<sub>2</sub> was taken for lytic reaction.

### *Immune lysis of liposomes*

The lytic reaction was carried out in 4  $\times$  4 mm fluorimeter cuvettes in a Hitachi-Perkin Elmer spectrofluorometer MPF III. Ten  $\mu$ l of liposome suspension in buffer A (about 8 nmoles of PC) were added to 300  $\mu$ l of buffer B, at 36°C in the reaction cuvette. Antiserum was added thereafter and the mixture was incubated for 5 min. Complement was added last and the increase in fluorescence at 520 nm (excitation wavelength 358 nm) was immediately recorded. After a plateau was reached, 5  $\mu$ l of 15% solution of Triton X-100 in water were added, resulting in disruption of the liposomes and total release of the fluorophore. The fluorescent signal thus obtained was taken as the 100% release value. Standard reaction mixture contained 15 to 20  $\mu$ l anti-GM<sub>2</sub> and 10  $\mu$ l human complement (unless shown otherwise).

### *Calculations*

The release curves were analysed in a Hewlett-Packard 9820A calculator equipped with a digitizer model No. 9864A and a plotter (No. 9862A). The

curves were corrected for the time dependent changes in fluorescence of components in the sera (antiserum and complement source). The release of ANTS and the rate of release (expressed as percent release per min) were calculated and plotted by the calculator plotter. An example to the form in which the results are obtained is given in fig. 1.

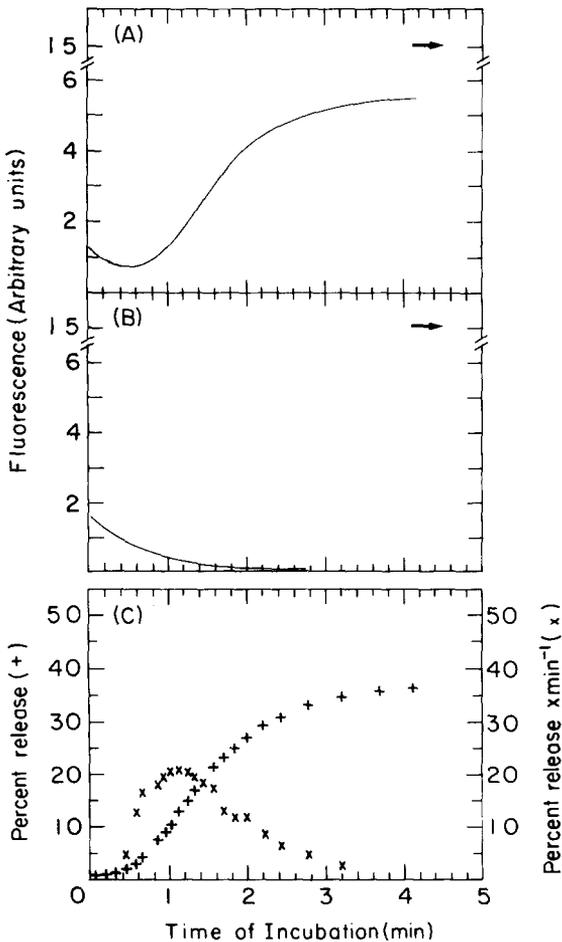


Fig. 1. Antibody-mediated complement lysis of GM<sub>2</sub> lysosomes. The reaction mixture consisted of 10  $\mu$ l of GM<sub>2</sub> liposomes (0.9 nmole GM<sub>2</sub>) 20  $\mu$ l anti-GM<sub>2</sub> and 10  $\mu$ l human complement. The arrows indicate the fluorescence obtained after the addition of Triton X-100, which was considered as 100% lysis. A) Fluorescence as a function of time after the addition of complement. B) The same as A but in the absence of fluorescent liposomes. C) The curves of lysis and the rate of lysis after correction for fluorescence changes of the complement and antiserum.

## RESULTS

*Titration of anti-GM<sub>2</sub>*

All the immunized animals produced antibodies to GM<sub>2</sub>, as concluded from the capacity of their sera to lyse, together with complement, GM<sub>2</sub>-containing liposomes. However, the responses of the animals which were injected with GM<sub>2</sub> and BSA were lower than those of the animals injected with GM<sub>2</sub> alone. Titration of anti-GM<sub>2</sub> was done by following the immune lysis of GM<sub>2</sub>-fluorescent liposomes caused by increasing amounts of anti-serum. The series of curves obtained enables the determination of both maximal release of ANTS and the maximal rate of release. Fig. 2 shows the rate of lysis of GM<sub>2</sub>-liposomes as a function of the amount of anti-GM<sub>2</sub> added (total volume of added serum was brought to 20  $\mu$ l with non-immune serum). In this study the amount (15–20  $\mu$ l) of anti-GM<sub>2</sub> added to a standard reaction mixture was in a region where a good linear correlation was found between the amount of anti-GM<sub>2</sub> and the rate of lysis. Different preparations of anti-GM<sub>2</sub> in different experiments gave similar values (40–60% maximal release per minute). It should be mentioned that the maximal release (at plateau) obtained under these conditions was up to 70–75% of the total entrapped ANTS. The specificity of the reaction was established since: a) Non-immune serum which was added to the anti-GM<sub>2</sub> to bring up the volume of added serum to 20  $\mu$ l, did not cause lysis, even after the addition of complement. b) The lysis of liposomes containing only MIX A lipids by anti-GM<sub>2</sub> and complement was found to be negligible (maximal rate of release <3% release per minute) as compared to the rate of lysis of the GM<sub>2</sub>-

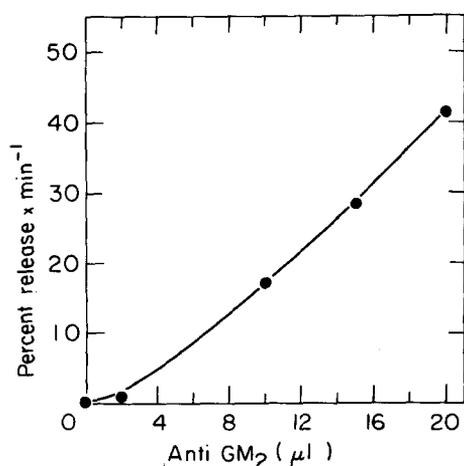


Fig. 2. Specific lysis of GM<sub>2</sub> liposomes by various amounts of anti-GM<sub>2</sub> antiserum. The assay consisted of 10  $\mu$ l of GM<sub>2</sub> liposomes (0.9 nmole GM<sub>2</sub>), 0 to 20  $\mu$ l of anti GM<sub>2</sub> (brought to 20  $\mu$ l with non-immune serum) and 10  $\mu$ l of complement.

containing liposomes which is described above. c) The antisera by themselves did not show any lytic capacity but required the presence of fresh active complement source.

It should be mentioned however, that low, non-specific lysis could be obtained when the antiserum was not clear and had protein aggregates. Upon the removal of such aggregates by centrifugation most of the non-specific lysis was abolished.

### *Titration of complement*

In most of the experiments reported here, human complement has been used. However, comparison had been made between this complement source, and commercially available guinea-pig complement (Difco). Complement (10  $\mu$ l), either fresh or heat-inactivated (40 min at 56°C) were added to GM<sub>2</sub>-liposomes, sensitized with rabbit anti-GM<sub>2</sub> (20  $\mu$ l), and the lytic reaction recorded (fig. 3). As depicted in the figure, the human complement was found to be much more active than that of the guinea pig, giving a lytic reaction with maximal rate of over 50% release per minute. The guinea-pig complement which was used here gave only a release of 10% per minute or less. Heating of both complement preparations (C and D in fig. 3) resulted in an almost complete abolishment of the lytic process.

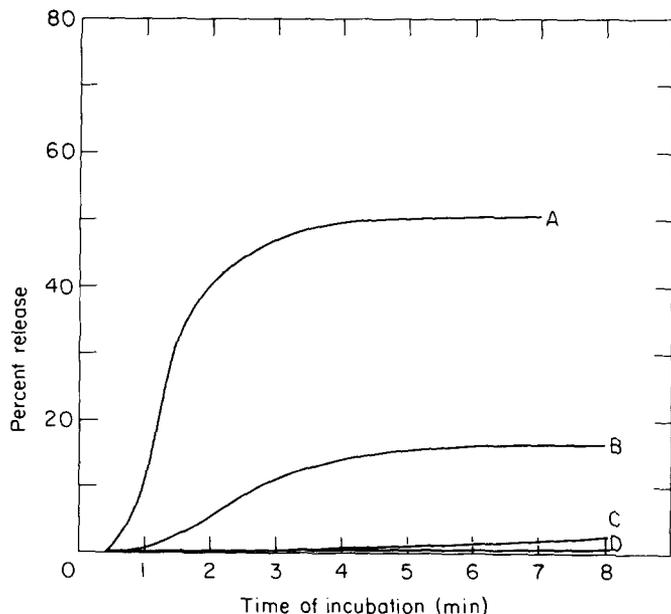


Fig. 3. Immune lysis of GM<sub>2</sub> liposomes (10  $\mu$ l, 0.9 nmole GM<sub>2</sub>) by anti-GM<sub>2</sub> (20  $\mu$ l) and various complement preparations (10  $\mu$ l). A) Fresh human complement. B) Commercial guinea-pig complement. C) Heat-inactivated (40 min, 56°C) human complement. D) Heat-inactivated (40 min, 56°C) guinea-pig complement.

The effect of complement concentration on the antibody-mediated lysis of  $GM_2$ -liposomes is given in fig. 4. As is evident from the figure a correlation is found between the concentration of complement and the observed lysis. Higher concentrations of human complement than those used in this study are undesirable because of its high endogenous fluorescence.

*The effect of dispersing  $GM_2$  in accessory lipids on its ability to inhibit the immunolysis of fluorescent  $GM_2$ -liposomes*

The ability of  $GM_2$ , dispersed in liposomes of various lipid compositions or as micelles in aqueous solution, to inhibit the immunolysis of fluorescent  $GM_2$ -liposomes was studied. The accessory lipid compositions tested were PC, PC + cholesterol and PC + cholesterol + DCP. The results are summarized in table 1. The strongest inhibition was obtained by liposomes containing PC + cholesterol + DCP (78.8%), but the accessory lipids by themselves show a high, nonspecific inhibition of about 25%. The best combination was  $GM_2$  in liposomes containing, in addition, only PC. The inhibitory capability of  $GM_2$  as a function of its dispersion in  $GM_2$ -PC liposomes was studied. Non-fluorescent liposomes (10  $\mu$ l) containing a constant amount (4.5 nmole) of  $GM_2$  and various concentrations of PC (from 0 to 90% on weight basis) were incubated with fluorescent  $GM_2$  liposomes (0.9 nmole  $GM_2$  in MIX A lipids) prior to the addition of anti- $GM_2$  and complement. The extent of inhibition of lysis of the ANTS-DPX-containing liposomes was calculated from controls which had not been inhibited. The results are summarized in Fig. 5. Liposomes consisting of PC only, without any  $GM_2$ , exerted low, non-specific inhibition of about 10% of the lytic reaction. Solution of  $GM_2$  alone had a relatively low inhibitory capacity of about 32%. However, when  $GM_2$

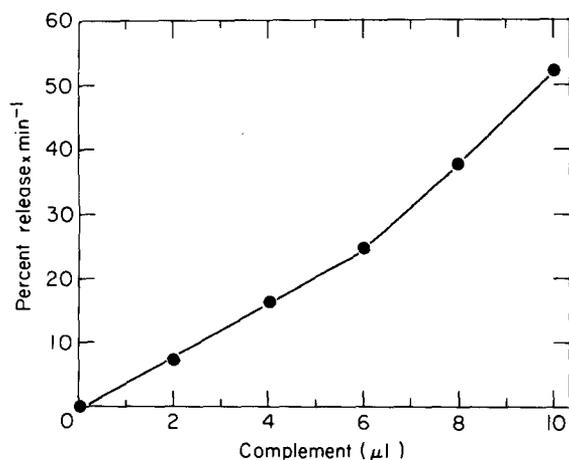


Fig. 4. Immune lysis of  $GM_2$  liposomes (10  $\mu$ l, 0.9 nmole  $GM_2$ ) by anti- $GM_2$  (20  $\mu$ l) and various amounts of human complement. The correction for fluorescence changes in the complement solution (see fig. 1) was performed for each point.

TABLE 1

The effect of dispersing GM<sub>2</sub> in accessory lipids on its ability to inhibit the immunolysis of fluorescent GM<sub>2</sub>-liposomes <sup>a</sup>.

Composition of inhibitory liposome (nmoles/assay)				Rate of release (% release per min)	Percent inhibition
PC	Chol	DCP	GM <sub>2</sub>		
40	—	—	4.5	24	74
40	—	—	—	79	12
40	30	—	4.5	33	63
40	30	—	—	75	16
40	30	0.8	4.5	19	79
40	30	0.8	—	67	25
—	—	—	—	90	0

<sup>a</sup> The inhibitory liposome suspensions (10  $\mu$ l) were mixed with the fluorescent liposomes (containing 0.9 nmoles GM<sub>2</sub> per assay) in 300  $\mu$ l buffer B prior to the addition of anti-GM<sub>2</sub> (15  $\mu$ l) and complement (10  $\mu$ l).

was mixed with PC, a marked enhancement was found in the inhibitory capability reaching a plateau at PC concentration of over 75%. This liposome composition was used in the rest of the inhibition studies.

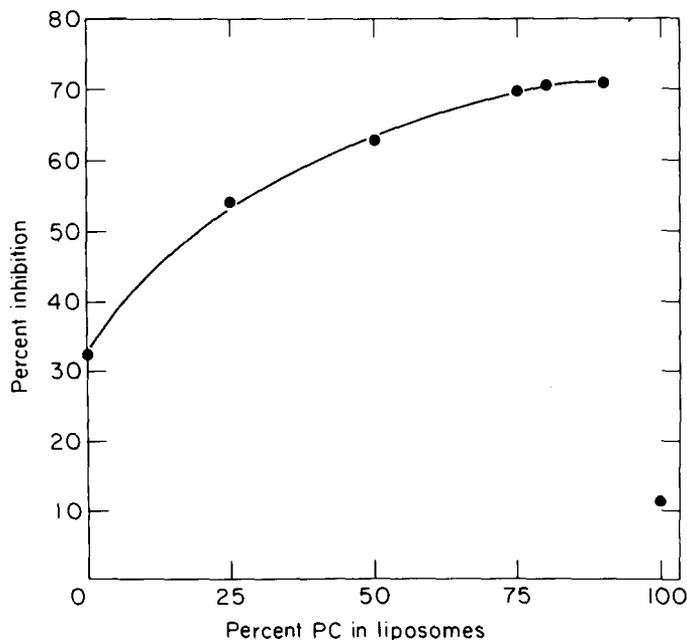


Fig. 5. Inhibition of lysis of GM<sub>2</sub>-fluorescent liposomes (10  $\mu$ l, 0.9 nmole GM<sub>2</sub> in MIX A) by non-fluorescent liposomes containing 4.5 nmole GM<sub>2</sub> and up to 90 nmoles of PC. The values of 0 and 100% PC represent the inhibition by GM<sub>2</sub> micelles and pure PC liposomes respectively.

*Quantitative determination of GM<sub>2</sub> by inhibition of the immune lysis of GM<sub>2</sub> liposomes*

Unlabeled liposomes consisting of PC-GM<sub>2</sub> (75 : 25 w/w) in increasing amounts were allowed to compete with fluorescent GM<sub>2</sub> liposomes on the binding of anti-GM<sub>2</sub> antibodies and complement. Varying volumes (up to 10  $\mu$ l) of a suspension of non-fluorescent liposome (containing 0.9  $\mu$ mole GM<sub>2</sub> per ml) were mixed with GM<sub>2</sub> liposomes containing ANTS-DPX prior to the addition of antibodies and complement. The inhibition by liposomes containing PC only in the same concentrations was considered as 100% activity controls in the calculations of the individual inhibition values (namely the percent decrease in rate of liberation of ANTS-DPX from the GM<sub>2</sub>-liposomes). The results are presented in fig. 6. A linear inhibition of liposome lysis was obtained for up to about 0.8 nmoles of GM<sub>2</sub> (fluorescent liposomes contained 0.9 nmoles GM<sub>2</sub> per assay). Addition of higher amounts of inhibitory liposomes resulted in an increase in the extent of inhibition up to 70–80% (in different experiments). Complete inhibition could not be obtained in this system. As seen in fig. 6, a value of about 50% inhibition

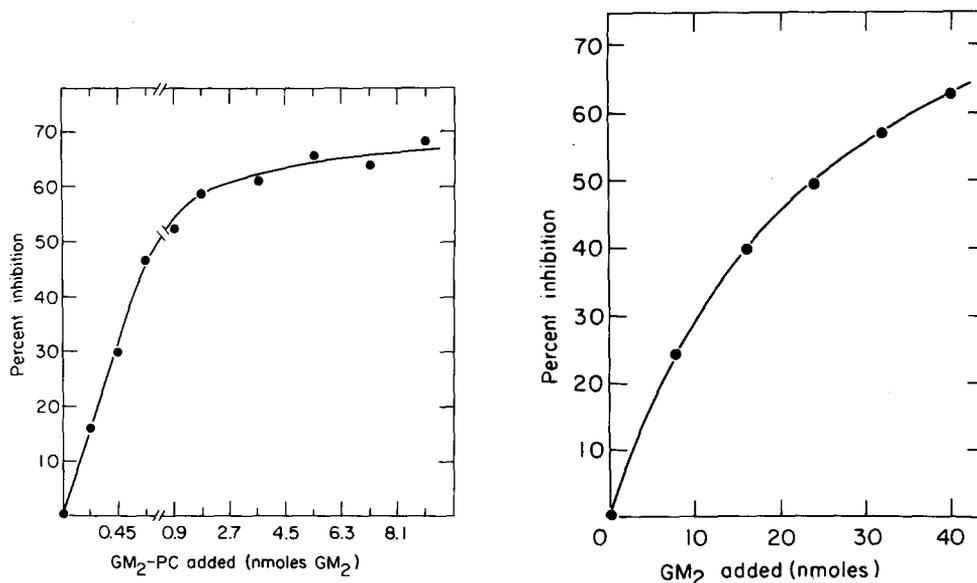


Fig. 6. Inhibition of lysis of GM<sub>2</sub> fluorescent liposomes (10  $\mu$ l, 0.9 nmole GM<sub>2</sub> in MIX A) by various amounts of non-fluorescent GM<sub>2</sub>-PC liposomes (25 : 75 w/w). Different amounts, up to 10  $\mu$ l of inhibitor (from stock solution containing 900 nmole/ml) were mixed with the fluorescent liposomes prior to the addition of antibody and complement.

Fig. 7. Inhibition of antibody-mediated complement lysis of GM<sub>2</sub>-fluorescent liposomes (10  $\mu$ l, 0.9 nmole GM<sub>2</sub> in MIX A) by pure GM<sub>2</sub> micelles in the various concentrations. The inhibitor was added in a volume of 10  $\mu$ l prior to the addition of anti-GM<sub>2</sub> (20  $\mu$ l) and complement (10  $\mu$ l).

was obtained when equal amounts of GM<sub>2</sub> were present in the test liposomes and in the inhibitory liposomes. The size of the inhibitory GM<sub>2</sub> liposomes did not seem to affect their inhibitory capability, since sonicated liposome suspensions (which were apparently transparent) were inhibitory to the same extent as non-sonicated liposomes. Moreover, identical inhibition values were obtained also by 0.1 to 1 nmoles of GM<sub>2</sub> dispersed in a fixed amount (10 nmoles) of PC.

The inhibition of the immunolysis of fluorescent GM<sub>2</sub>-liposomes by varying amounts of GM<sub>2</sub>-micelles was also studied. The inhibition curve is shown in fig. 7. It can be concluded from the data presented in fig. 6 and 7 that competition of the immune lysis can be achieved by GM<sub>2</sub> micelles, but it requires much higher concentrations of GM<sub>2</sub> than those needed when GM<sub>2</sub>, dispersed in PC-liposomes is used for inhibition. About 20 nmole of micellar GM<sub>2</sub> were required for 50% inhibition of the lysis of the fluorescent liposome (containing 0.9 nmoles GM<sub>2</sub>), in contrast to about 0.8–1.0 nmole of GM<sub>2</sub> incorporated in PC liposomes.

The possibility that passive sensitization may take place in the inhibition systems, namely the incorporation or absorption of GM<sub>2</sub>, originally present in the inhibitor solution into the fluorescent liposomes, was tested and excluded; fluorescent liposomes composed of MIX A lipids only were incubated with PC-GM<sub>2</sub> liposomes (0.5 to 5 nmoles GM<sub>2</sub>) or with 30 nmoles of GM<sub>2</sub> for up to 60 min at 37°C. Antibodies and complement were added sequentially thereafter, and the reaction recorded. In all the experiments no response was obtained, indicating that no apparent passive incorporation of GM<sub>2</sub> into the test liposomes took place.

TABLE 2

Inhibition of antibody-mediated complement lysis of GM<sub>2</sub> liposomes by structurally related sphingolipids.

Inhibitory liposome <sup>a</sup>	Rate of release (percent release/min)	Percent Inhibition
none	55	0
100%PC	50	8
9% GM <sub>2</sub> , 91% PC	28	49
9% sulfatide, 91% PC	39	20
9% globoside, 91% PC	54	2
9% glucosyl ceramide 91% PC	56	0
9% galactosyl ceramide 91% PC	43	21
9% sphingomyelin, 91% PC	52	5

<sup>a</sup> Liposome suspension (10 μl) containing 1 nmole of sphingolipid and 10 nmoles of PC were mixed with the standard GM<sub>2</sub>-containing fluorescent liposomes (0.9 nmole GM<sub>2</sub> in MIX A) prior to the addition of anti-GM<sub>2</sub> (20 μl) and complement (10 μl).

*Immunological cross-reaction between GM<sub>2</sub> and other glycolipids*

In order to study the antigenic cross reactivities of various lipids, structurally related to GM<sub>2</sub>, liposomes containing 1 nmole of the various lipids (GM<sub>2</sub>, sulfatide, globoside, glucosyl ceramide, galactocerebroside and sphingomyelin) dispersed in 10 nmoles of PC were allowed to compete with GM<sub>2</sub> fluorescent liposomes (containing 0.9 nmoles of GM<sub>2</sub> per assay) for the antibodies and complement. The inhibition values are given in table 2. Similar results were obtained with 4 different anti-GM<sub>2</sub> antisera, with only limited variation. It can be concluded that out of the materials tested only the sulfatide and galactocerebroside share antigenic similarity with GM<sub>2</sub>.

## DISCUSSION

The experiments described above illustrate the possibility of using the inhibition of immune lysis of liposomes for the quantitative determination of a lipid antigen and for the comparison of its antigenicity with that of other, structurally related lipids.

Theoretically, two alternative approaches could be taken for the analysis of the interaction of lipid antigens and their respective antibodies. The first is a direct analysis of the antibody-mediated complement lysis of liposomes containing the fluorophore in their entrapped aqueous volume, and the lipid antigen in their lipid shell. The second possibility, the one which was studied here, is based on the inhibition of the specific lysis of antigen-containing fluorescent liposomes by the lipid antigen which is added in the form of non-fluorescent liposomes or in micelles. The latter approach seems to have some advantages over the first one, since as an inhibitor the lipid antigen can be mixed with any desired accessory lipids which will not have antigenic cross-reactivity with the studied antigen and will enable optimal access of the antigenic determinants to the antibodies, but will not necessarily be suitable for entrapment of the fluorophore or for complement lytic action. The inhibitor may also be added in micellar form into which no fluorescent marker can be introduced.

An important requirement for the full expression of antigenic determinants of GM<sub>2</sub> is its 'dilution' in membrane which is built of 'accessory lipids'. The best results were obtained in our case, with PC as the dispersing lipid. Optimal 'dilutions' of the GM<sub>2</sub> required at least a 3-fold excess on weight basis of PC over GM<sub>2</sub>, which is about 6 fold excess on molar basis. This explains also why micelles of GM<sub>2</sub> in buffer were not efficient inhibitors to the GM<sub>2</sub>-liposomes lysis.

We have used here as marker-containing liposomes, non-sonicated vesicles which are, presumably multilamellar; however, the maximal lysis obtained in our system resulted in maximal release of over 70% of the entrapped marker (in another antigen-antibody system maximal release values of over 90%

were obtained). This suggests that complement lysis may not be restricted to the outer lipid shell only, but may disrupt also internal layers. We found also that the size of the inhibitory GM<sub>2</sub> liposomes did not seem to affect their inhibitory capability, since sonication of the multilamellar (vortexed) liposomes did not alter their inhibitory effect.

The results presented in fig. 6 show that the concentration of GM<sub>2</sub> can be determined quantitatively in the range where the inhibition of the immunolytic reaction is linearly dependent on GM<sub>2</sub> concentration (as GM<sub>2</sub>-PC liposomes). The sensitivity of the method is dependent on the quality of the antibodies, complement and the fluorometer used. Under optimal conditions the sensitivity for the detection of GM<sub>2</sub> is high: 0.1–0.8 nmole (fig. 6).

Beside the quantitative determination of lipophilic antigens the presented technique can also be useful in studying the immunological cross-reactivity between various antigens associated with lipid moieties. The antigenic cross-reactivity between GM<sub>2</sub> and structurally related glycolipids had been tested in the experiments described in table 2. It was found that only the sulfatide and the galactocerebroside have common antigenic properties with GM<sub>2</sub>. Further information on the anti-GM<sub>2</sub> antibodies can be obtained by studying the inhibition of the immunolysis reaction by soluble sugars and oligosaccharides, which are structurally related to the hydrophilic portion of GM<sub>2</sub>.

The criterion for evaluation of the lytic reaction, used throughout this study, was the maximal rate of release of the entrapped fluorophore into the external liposomal solution. In our system (results are not shown here) this parameter was found to be more sensitive and reproducible, and to represent better the number of 'hits' or holes, formed in the liposome membrane during the immunolytic reaction, than the total release which is commonly employed (Haxby et al., 1968; Alving et al., 1969; Kinsky et al., 1969; Knudson et al., 1971; Six et al., 1973). A linear correlation between the concentration of antibodies (or complement) and the rate of release was found in the range of concentration wider than that which was obtained with the total release parameter. The difference between these two parameters was remarkable, especially when high concentrations of either antibodies or complement were used. In these cases linearity could still be obtained between the concentration of serum (or C') and the rate of lysis although the total release reached a plateau. Similar results were obtained when the immune lysis of liposomes containing sheep-erythrocyte lipid extract, by anti-sheep erythrocyte antibodies was studied (Smolarsky et al.).

Results of preliminary experiments show that the method presented here may serve for the quantitative determination of a variety of antigens with lipophilic properties including naturally occurring lipids, blood-group substances and cell-membrane proteins. It may well be used also for the study of the mode of interaction of these antigens with antibodies and the subsequent complement damage to the membrane.

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