ANTILIPOSOME ANTISERA ACTIVITY AGAINST NEGATIVELY CHARGED PHOSPHATE AMPHIPHILS +

S.W. Chan, C.T. Tan, and J. Carleton Hsia

Department of Pharmacology
Faculty of Medicine
University of Toronto
Toronto, Canada M5S 1A8.

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SUMMARY. Complement-immune lysis of liposomes loaded with water-soluble spin label 2,2,6,6-tetramethyl-piperidinoxy-choline chloride (TEMPO-choline) was used to measure specificity of rabbit antisera generated by complete adjuvant with liposomes consisting of sphingomyelin; cholesterol; dicetylphosphate; 5-N-thyroxine-2,4-dinitrophenyl-phosphatidylethanolamine (T_4-Dnp-PE) in the ratio 2.0:1.5:0.2:15. Antisera so generated induced complement lysis of the liposomes containing negatively charged amphiphils. No immune lysis of positively charged liposome was observed. This antiliposome specificity is retained in partially purified antibodies.

Immunization with natural membranes can elicit antibodies against lipid antigens (1-3). The pioneering work of Kinsky and co-workers showed that sensitization of liposomal membranes with N-substituted phosphatidylethanolamine derivatives not only can render them immunologically sensitive (4) but also can provoke antibodies against them (5,6,7,8,9).

In an attempt to obtain antibodies to thyroxine, using liposomes as carriers we immunized rabbits with a liposome preparation containing thyroxine-conjugated phosphatidylethanolamine in complete adjuvant. Preliminary results showed that anti-thyroxine antibodies can be generated. However, during analysis of the anti-thyroxine antisera, we noted by chance that, even without sensitization by thyroxine-conjugated phosphatidylethanolamine, such antisera can induce specific immune lysis of several types of liposomes containing negatively charged amphiphils.

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MATERIALS AND METHODS

Sphingomyelin (SP), dicetylphosphate (DCP), and egg phosphatidylcholine (EPC) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. Cholesterol (CHL) was twice recrystallized (m.p., 148.0-148.5). Cardiolipin (CL), egg phosphatidic glycerol (EPG) and dipalmitoyl phosphatidic acid (PA) were purchased from Serdary Research Lab. Inc., London, Ontario, Canada; and stearylamine (SA) from Grand Island Biological Co., Grand Island, N.Y., U.S.A. Rabbit antisera (A and B; see Table 1) were produced by immunizing 2 animals with SP/CHL/DCP/T4-Dnp-PE (2:1.5:0,2:0,15) liposomes with complete Freund's adjuvant. Partially purified specific antibodies against negatively charged liposomes were isolated by according to the method of Alving and Richards (10). The antibodies first were absorbed to SP/CHL/DCP (2:1.5:0.2) liposomes and then were recovered, after washing the liposomes, by elution with 1M NaI.

SP (or EPC)/CHL/DCP (2.0:1.5:0.2) liposomes containing 2,2,6,6-tetramethyl-piperidinoxy-choline chloride (TEMPO-choline) (11) were prepared (4). In other liposome preparations, SA, CL,PA or EPG was substituted for DCP. Release of the TEMPO-choline from liposomes was recorded on a Varian E-6 X-band electron spin resonance (ESR) spectrometer at 20°C (12).

RESULTS AND DISCUSSION

Figure 1 shows specificity of the antisera to SP/CHL/DCP liposomes in a representative assay. No specific immune lysis of liposomes was detectable when either rabbit antisera or guinea-pig serum-complement also was present (spectra B and C). Specific immune lysis occurred only when both antisera and serum-complement were present (D). Furthermore, SP/CHL/DCP liposomes without entrapped spin labels inhibited immune lysis (E). Complete lysis is spectrum F in the figure.

Spin-label release was expressed as the percentage of complete lysis of liposomes 30 min after addition of complement (L_{30}). Immune liposomal lysis by antisera from 2 rabbits were membrane-charge specific: only negatively charged liposome preparations were sensitive (Table 1). Similar results were obtained to the partically purified antibodies. Non-immunized rabbit antisera were inactive (not shown).

Our findings indicate the presence antibodies in the rabbit's sera against the negatively charged phosphate amphiphils of the liposomal preparations with which the animals had been injected. This may explain the nonspecific lysis of unsensitized liposomes (SP/CHL/DPC) by guinea-pig antisera against liposomes with a Dnp sensitizer (6).

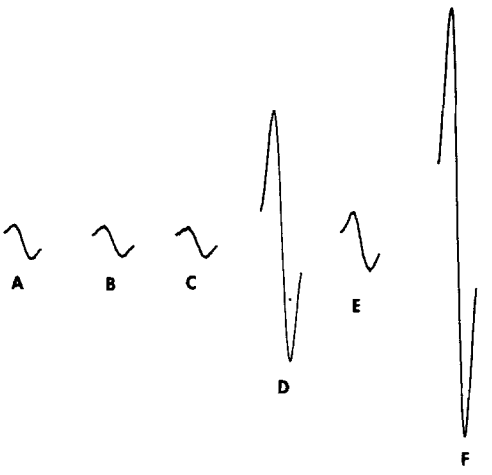

Figure 1. ESR intensity of the high-field signal (M_{-1}) of 2,2,6,6-tetramethyl-piperidinoxyl-choline chloride (TEMPO-choline) encapsulated in liposomes and after released from liposomes into salt solution (0.15 M NaCl, 1 mM $MgCl_2$, and 0.15 mM $CaCl_2$). A (control), trapped in liposomes; B, as for A plus guinea-pig serum; C, as for A plus rabbit antiserum; D, as for A plus both rabbit antiserum and guinea-pig serum; E, as for D plus liposomes without trapped TEMPO-choline; F, complete lysis of A under one rapid freezing in liquid nitrogen and thawing. Details of assay protocol was the same as described in Table I.

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TABLE I

Immune Lysis of Liposomes Sensitized with Charged Amphiphils[a]

Liposomes (Charge)	L_{30} (% of complete Lysis)[b] Serum A	Serum B
SP/CHL/DCP (-)	55	54
EPC/CHL/DCP (-)	64	60
SP/CHL/EPG (-)	49	51
SP/CHL/PA (-)	47	50
SP/CHL/CL (-)	60	60
SP/CHL/SA (+)	<3	<3
EPC/CHL/SA (+)	<3	<3

[a]Negatively charged amphiphilic molecules (DCP, EPG, PA and CL)or positively charged (SA).

[b]The assay system contains: 0.2 µl liposomes, 0.5 µl or rabbit antiserum and 2 µl of guinea-pig serum, the final volume of 100 µl was balanced with salt solution (0.15 M NaCl, 1 mM $MgCl_2$, and 0.15 mM $CaCl_2$). In all cases, guinea-pig serum (as source of complement) was added at the final step to trigger complement lysis via classical pathway. The ESR measurement were the same as described in Fig. 1. The degree of lysis at 30' (L_{30}), after the addition of complement, was expressed in per cent of complete lysis over control (i.e. L_{30} = [D-B (or C)]/[F-B (or C)] x 100%, as shown in Figure 1).