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Human Hybridoma Lupus Anticoagulants Distinguish between Lamellar and Hexagonal Phase Lipid Systems*

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Antibodies to phospholipids may have important physiological and biological functions. Lupus anticoagulants represent a subclass of anti-phospholipid antibodies which are characterized by their ability to prolong the clotting time in *in vitro* coagulation assays measuring partial thromboplastin time (PTT) (Thiagarajan, P., Shapiro, S. S., and DeMarco, L. (1980) *J. Clin. Invest.* 66, 397–405). In the present study, we produced hybridomas by fusing lymphocytes from 13 systemic lupus erythematosus patients with the GM 4672 lymphoblastoid line. Of the resulting 67 hybridoma autoantibodies, 14 (21%) were found to prolong a modified PTT assay, and 11 of these antibodies were analyzed further. Competition experiments, using a modified PTT assay, demonstrated that hexagonal phase phospholipids, including natural and synthetic forms of phosphatidylethanolamine, were able to neutralize the lupus anticoagulant activity of all 11 hybridoma antibodies. In contrast, lamellar phospholipids, such as phosphatidylcholine and synthetic lamellar forms of phosphatidylethanolamine, had no effect on the anticoagulant activity. Thus, these antibodies are capable of recognizing phospholipids on purely structural criteria. The demonstration that anti-phospholipid antibodies are able to distinguish between different structural arrangements of phospholipid may have important implications regarding the immunoregulation of autoimmunity.

Antibodies to phospholipids represent a group of poorly characterized antibodies which may have important physiological and biological functions (1). These antibodies occur frequently in the sera of patients with a variety of autoimmune diseases, including systemic lupus erythematosus (SLE[1]) and related connective tissue disorders (2). The mechanism(s) responsible for the production of these autoantibodies remains unknown. Recently, it has been found that antibodies against commonly occurring membrane lipids such as phosphatidylcholine, sphingomyelin, and possibly cholesterol are ubiquitous in normal (nonimmunized) human sera (3). Apparently, normal tissue is protected against attack by anti-phospholipid antibodies, but again, the mechanism remains obscure. One possible mechanism for the induction and/or pathogenesis of these antibodies involves alterations in the phospholipid architecture of the cell membrane. Such an explanation would seem plausible in light of the ability of membrane lipids to assume a variety of structures in addition to the bilayer phase (4). In fact, in certain pathological conditions, non-bilayer lipid structures have been demonstrated *in vivo* (5). Although such structures could conceivably play a role in antibody induction or pathogenesis, this possibility requires a clear demonstration that anti-phospholipid antibodies exist which are able to distinguish different polymorphic forms of the same lipid.

Our approach to this problem has involved the use of human hybridoma lupus anticoagulants derived from patients with SLE. Lupus anticoagulants are anti-phospholipid antibodies (6, 7) which are defined by their ability to prolong the normal clotting time in *in vitro* coagulation assays measuring the partial thromboplastin time (PTT). In these assays, platelet membranes are replaced by brain cephalin, a lipid extract rich in phosphatidylethanolamine. In this study, we show that the ability of lupus anticoagulants to prolong PTT values can be inhibited by the presence of hexagonal phase, but not bilayer phase, phosphatidylethanolamine. Hexagonal phase phosphatidylethanolamine, in contrast to the familiar bilayer phase, consists of hexagonally packed cylinders of lipid where the cylinders are composed of a central aqueous channel toward which the polar head groups are oriented (4). Thus, we describe here the ability of lupus anticoagulant antibodies to distinguish between different structural configurations of the same chemical species of phospholipid.

MATERIALS AND METHODS

Preparation of Phospholipids—Egg phosphatidylethanolamine (PE), egg phosphatidylcholine, dioleoylphosphatidylethanolamine (dioleoyl-PE), dipalmitoylphosphatidylethanolamine (DPPE), monomyristoyl PE, and monopalmitoyl PE were purchased from Avanti Polar Lipids (Birmingham, AL). Bovine brain PE was from Supelco (Bellefonte, PA). These lipids were used without further purification. Another species of egg PE (egg PE(PC)) was derived from its respective phosphatidylcholine, employing the base exchange capacity of phospholipase D (8) and purified as previously described to greater than 99% pure, as detected by thin layer chromatography (9). Stock solutions were prepared as follows. A volume containing 4 mg of phospholipid in chloroform was placed in a round-bottom glass tube

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[1] The abbreviations used are: SLE, systemic lupus erythematosus; dDNA, denatured DNA; DPPE, dipalmitoylphosphatidylethanolamine; IgG, immunoglobulin G; PE, phosphatidylethanolamine; PE(PC), phosphatidylethanolamine derived from egg yolk phosphatidylcholine; PTT, partial thromboplastin time(s); Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

and the chloroform removed by evaporation under dry nitrogen. The dried lipids were resuspended in 1.0 ml of 20 mM Hepes buffer (150 mM NaCl, pH 7.5), hydrated at a temperature above their transition temperature for 1–2 h and stored at 4 °C. Phosphate concentrations were determined by the method of Bartlett (10).

NMR—^{31}P NMR spectra were obtained using a Bruker WP-200 FT-NMR spectrometer operating at 81.0 MHz for ^{31}P. Lipids were dispersed in 10 mM Tris/HCl, 100 mM NaCl, 10% v/v ^2H_2O, pH 7, buffer by extensive vortexing at room temperature except for DPPE which was heated to 70 °C before dispersal. Spectra were accumulated at 37 °C for up to 2000 transients employing a 7-μs 90° radiofrequency pulse, 20-kHz sweepwidth, and 0.8-s interpulse delay, in the presence of broadband proton decoupling. An exponential multiplication corresponding to 50-Hz line broadening was applied to the free induction decay prior to Fourier transformation.

Production of Human-Human Hybridoma Lupus Anticoagulants—Fifty ml of venous blood was obtained from 13 patients who satisfied the revised American Rheumatism Association criteria for the classification of SLE (11). The plasma of two of these patients had elevated partial thromboplastin times, and another patient was positive in the Venereal Diseases Research Laboratories test for syphilis. The peripheral blood lymphocytes were isolated on a Ficoll-Hypaque density gradient and were fused with the GM 4672 human lymphoblastoid cell line (IgG producing), obtained from the Cell Respository Institute of Medical Research, Camden, NJ, at a cell ratio of 1:1, using 44.4% polyethylene glycol. The fusion, plating, and cloning procedures have been described elsewhere in detail (12). All hybridoma antibodies were IgM immunoglobulins, as determined by a solid phase radioimmunoassay (13).

Detection of Hybridoma Lupus Anticoagulants—Hybridoma supernatants were tested for the production of lupus anticoagulants using a modified activated PTT assay (14). Fifty μl of hybridoma culture supernatant was diluted with an equal volume of freshly reconstituted Verify normal citrate (pooled normal human plasma, General Diagnostics, Scarborough, Ontario) in a 13 × 100-mm borosilicate glass tube. One hundred μl of a 1/64 dilution of activated Thrombofax (Ortho Diagnostics Inc., Raritan, NJ) in Hepes buffer, which had been prewarmed to 37 °C, was then added and the mixture incubated for 5 min in a 37 °C circulating water bath (Haake, Saddle Brook, NJ). Next, 100 μl of a 2 mg/ml solution of kaolin (Fisher Scientific, Fair Lawn, NJ) in 0.1 M Tris-HCl buffer, pH 7.6, was added and incubated for 5 min in the 37 °C water bath, with shaking at 2-min intervals. Finally, 100 μl of 0.025 M $CaCl_2$ was added to the mixture to initiate clotting. The tilt-tube technique was used in which the tube was gently tilted back and forth in the 37 °C water bath, until a clot formed. The time required to observe the first sign of a clot was recorded. All samples were tested in duplicate.

Selection of Hybridoma Lupus Anticoagulants—GM 4672 is the parent lymphoblastoid cell for all of the described hybridomas, and its culture supernatant (containing GM 4672 IgG) was used as the negative control in all PTT assays. It did not prolong the partial thromboplastin time relative to Verify normal plasma and, therefore, had no lupus anticoagulant activity. Sixty-seven hybridoma IgM supernatants with varying immunoglobulin concentrations (0.03–74.0 μg/ml), as determined by a solid phase radioimmunoassay, were examined (13). Fifty-three of these gave PTT values below or equal (±1.2 s) to that of the GM 4672 control (mean PTT = 60 s). In our sampling of 67 hybridoma antibodies, an antibody was defined as having anticoagulant activity only if its PTT exceeded the PTT of the GM 4672 control by greater than 5 S.D. (6 s) (15). Fourteen antibodies satisfied this criterion, but only 11 were available in sufficient quantities to be analyzed further. Hybridoma antibody 1500 was selected as representative of the 53 antibodies lacking lupus anticoagulant antibody activity. This hybridoma supernatant, which had an IgM concentration equivalent to the mean concentration of the 11 lupus anticoagulant antibody supernatants (12 μg/ml), was used as the IgM anticoagulant-negative control in all of the PTT assays.

Titration of the Inhibition of the Anticoagulant Activity of a Hybridoma Lupus Anticoagulant after Preincubation with Egg PE at 37 °C—Dilutions of egg PE or egg PE(PC) containing between 1 and 134 nmol of phosphorus were added to one of the hybridoma antibodies exhibiting lupus anticoagulant activity (antibody 824, 0.75 ng) and preincubated for 10 min at 37 °C. In one set of controls, anticoagulant 824 was incubated with Hepes buffer, giving rise to maximum partial thromboplastin times (PTT_{max}). Controls without anticoagulant activity included GM 4672 IgG or 1500 IgM incubated with buffer, egg PE, or egg PE(PC), giving rise to minimum partial thromboplastin times (PTT_{min}). All of these mixtures were tested for lupus anticoagulant activity, employing the PTT assay as described above. The ability of the phospholipids tested to inhibit the lupus anticoagulant activity was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{PTT_{max} - PTT(824 + \text{phospholipid})}{PTT_{max} - PTT_{min}} \times 100\%$$

In some cases, phosphorylethanolamine, monomyristoyl PE, and monopalmitoyl PE were used in place of egg PE or egg PE(PC).

Titration of the Inhibition of the DNA-binding and Rheumatoid Factor Activities of Hybridoma Antibodies by DNA, Immunoglobulin, and Hexagonal and Lamellar PE—Dilutions of potential inhibitors (denatured DNA (dDNA), IgG, dioleoyl-PE, or DPPE), containing between 0.01 and 100 nmol of phosphorus, were added to hybridoma anti-DNA antibody 1206 (for the dDNA-binding assay) and to hybridoma rheumatoid factor antibody 18141 (for the rheumatoid factor assay) and preincubated for 1 h at 37 °C. Duplicate tubes containing buffer mixed with antibody served as controls for maximal binding in the absence of competitor. 75 μl of the mixture was then added, in duplicate, to polystyrene test tubes coated with either dDNA (2.5 μg/ml) (dDNA-binding assay) (13) or the Fc fragment of human IgG (2.5 μg/ml, rheumatoid factor assay) (16). The tubes were incubated for 1 h at 37 °C, for the anti-dDNA assay, and overnight at 4 °C, for the rheumatoid factor assay, and then washed three times with the assay buffer (0.1 M KPO_4, pH 7.0, containing 0.1% bovine serum albumin and 0.01% Tween 20). 100 μl of ^{125}I-radiolabeled rabbit anti-human IgM (100,000 cpm/tube) was then added and incubated overnight at 4 °C. The tubes were again washed three times with buffer and counted for bound ^{125}I radioactivity in a γ counter (Beckman Instruments). The results, expressed as percentage inhibition of antibody binding were calculated using the following formula.

$$\% \text{ Inhibition} = \frac{\text{binding (without inhibitor)} - \text{binding (with inhibitor)}}{\text{binding (without inhibitor)}} \times 100\%$$

Preincubation of Different Phospholipid Phase Systems with Lupus Anticoagulants at 37 °C: Effects on Partial Thromboplastin Times—The effects of different phospholipid systems on the partial thromboplastin times of the 11 human hybridoma lupus anticoagulants were assessed using a slight modification of the PTT assay, in which equal volumes of hybridoma culture supernatant and phospholipids were mixed and incubated for 10 min in a 37 °C water bath. Fifty microliters of this mixture was then added to 50 μl of Verify normal citrate, in duplicate, and the rest of the PTT assay was performed exactly as described above. Controls in each assay included the GM 4672 and 1500 supernatants diluted 1:1 with buffer containing phospholipid and each hybridoma supernatant diluted 1:1 with Hepes buffer.

Preincubation of Egg PE(PC) and Egg PE with Hybridoma Lupus Anticoagulants at 43 °C: Effects on Partial Thromboplastin Times—Egg PE(PC) and egg PE were hydrated in Hepes buffer at 37 °C, and aliquots were heated at 43 °C for 1 h. The preincubation of the phospholipids or buffer with the lupus anticoagulants or control antibodies was performed as above, except that the 10-min preincubation step was done at 43 °C. Controls in which the lupus anticoagulant was preincubated with the same phospholipids or buffer at 37 °C were included in each experiment. The phospholipid in the 43 °C preincubation mixture was removed by filtration through a 13-mm 0.22-μ Millipore filter unit, which had been preheated to 45 °C. The filtrates were then assayed in the regular PTT assay. Lupus anticoagulant antibodies, preincubated with the same phospholipids at 37 °C and filtered through unheated 0.22-μm filter units, served as controls.

Statistical Analysis—Statistical analysis of the differences between PTT values in the absence and presence of different phospholipid systems was performed using the sign test (17). Comparisons were always made with buffer (no phospholipid) controls run in the same experiment.

RESULTS

Phospholipid Phase Assignments—The polymorphic phase preferences of the phospholipids used were determined employing the ^{31}P NMR technique (4). Asymmetric ^{31}P NMR spectra exhibiting a low field shoulder and high field peak are

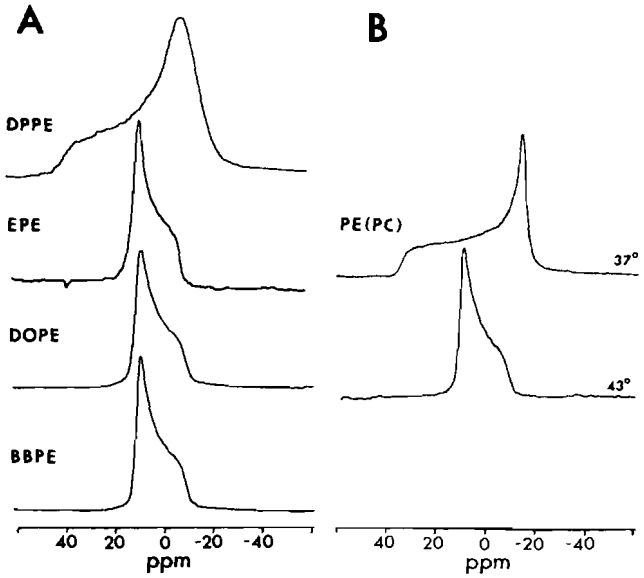

FIG. 1. **81.0-MHz ^{31}P NMR spectra of aqueous dispersions of various species of phosphatidylethanolamines.** *A*, DPPE, egg yolk phosphatidylethanolamine (*EPE*), dioleoylphosphatidylethanolamine (*DOPE*), and bovine brain phosphatidylethanolamine (*BBPE*) at 37 °C. *B*, phosphatidylethanolamine derived from PE(PC) at 37 and 43 °C.

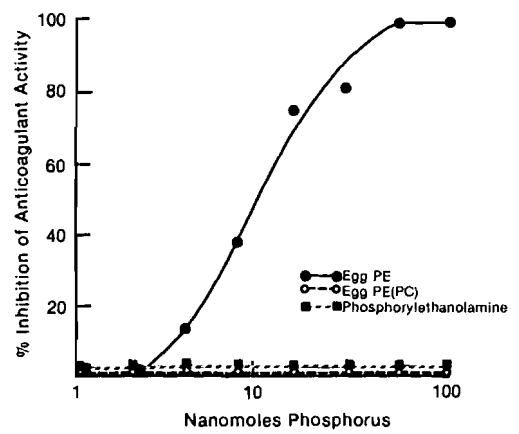

FIG. 2. **Titration of the inhibition of the anticoagulant activity of antibody 824 after preincubation with egg PE, egg PE(PC), or phosphorylethanolamine at 37 °C for 10 min.** At this temperature, egg PE is hexagonal and egg PE(PC) is lamellar. Controls included anticoagulant antibody 824 + buffer, giving rise to PTT_{max} = 71 s, as well as controls without antibody 824, containing GM 4672 IgG or 1500 IgM incubated with buffer, phospholipid, or phosphorylethanolamine, which gave rise to mean PTT_{min} = 61 s ± 1. The ability of the phospholipids to inhibit the lupus anticoagulant activity was calculated using the formula,

$$\% \text{ inhibition} = \frac{PTT_{max} - PTT(824 + \text{phospholipid})}{PTT_{max} - PTT_{min}} \times 100\%.$$

characteristic of bilayer phospholipids, whereas phospholipids in the hexagonal (H_{II}) organization give rise to spectra with reversed asymmetry which are approximately a factor of two narrower (4). As shown in Fig. 1*A*, DPPE assumes a bilayer organization at 37 °C, whereas dioleoyl-PE, bovine brain PE, and egg PE are in the hexagonal organization at these (and higher) temperatures. Egg PE(PC), which undergoes a bilayer to hexagonal (H_{II}) transition as the temperature is increased through 40 °C, adopts the bilayer phase at 37 °C and the hexagonal phase at 43 °C (Fig. 1*B*).

Titration of the Inhibition of the Anticoagulant Activity of a Hybridoma Lupus Anticoagulant by Egg PE at 37 °C—A titration curve of the inhibition of the anticoagulant activity of hybridoma lupus anticoagulant 824 by egg PE is shown in Fig. 2. Inhibition of the anticoagulant activity by egg PE, which adopts the hexagonal phase at 37 °C, occurred when greater than 2.1 nmol of phosphorus was added and was complete at 68 nmol of phosphorus. On the other hand, egg PE(PC), which is lamellar and liquid crystalline at 37 °C, and phosphorylethanolamine, the polar head group of PE, failed to inhibit anticoagulant activity over the same range of concentrations. Monomyristoyl PE, monopalmitoyl PE, phosphatidylcholine, and phosphatidylinositol also did not inhibit the anticoagulant activity of this antibody (data not shown).

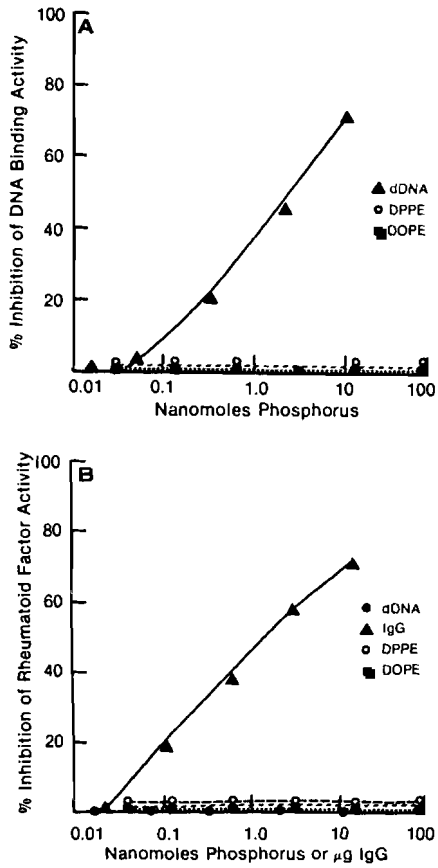

FIG. 3. **Titration of the inhibition of dDNA-binding activity of hybridoma antibody 1206 (*A*) and the rheumatoid factor activity of hybridoma antibody 18141 (*B*) after preincubation with dDNA, human IgG, dioleoyl-PE (*DOPE*), and DPPE at 37 °C for 1 h.** Controls included the hybridoma antibodies preincubated with buffer only (maximal binding in the absence of inhibitors). The percentage inhibition of maximal binding in each assay was calculated using the formula,

$$\% \text{ inhibition} = \frac{\text{binding (without inhibitor)} - \text{binding (with inhibitor)}}{\text{binding (without inhibitor)}} \times 100\%.$$

Titration of the Inhibition of the DNA Binding and Rheumatoid Factor Activities of Hybridoma Antibodies by DNA, Immunoglobulin, and Hexagonal and Lamellar PE—In order to ensure that hexagonal PE was not inhibiting the lupus anticoagulant activity by nonspecific interactions with hybridoma IgM antibodies, similar competition experiments using hexagonal and lamellar PE and other IgM hybridoma antibodies were performed in two other assay systems.

Fig. 3*A* shows the inhibition of the binding of hybridoma antibody 1206 to denatured DNA (dDNA) by dDNA, dioleoyl-PE, and DPPE. Seventy per cent inhibition of the DNA-

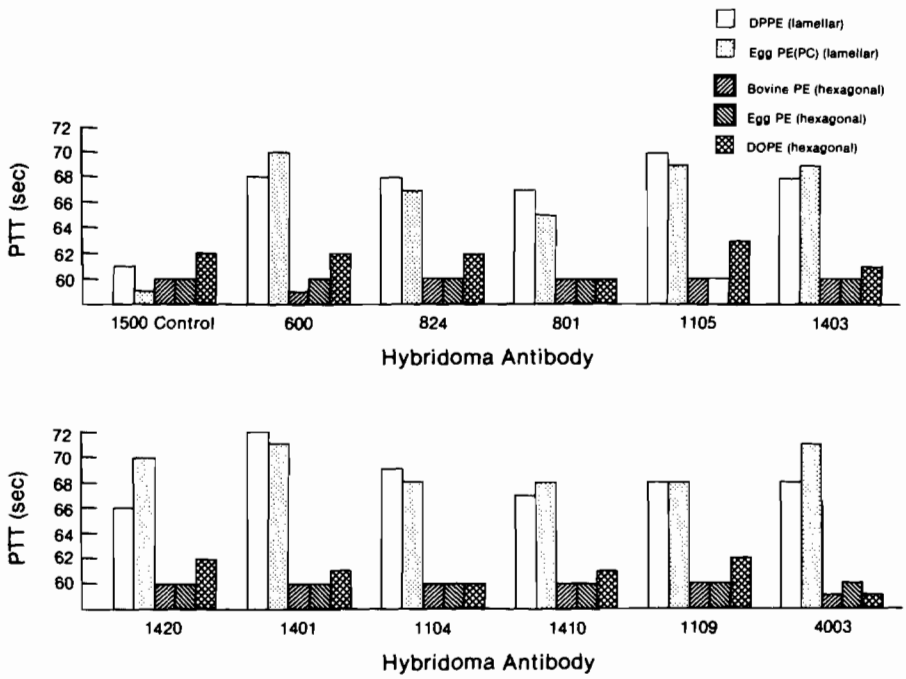

FIG. 4. **Effects of preincubation of different phospholipid phase systems with hybridoma lupus anticoagulants at 37 °C.** Hybridoma antibodies, including the 1500 IgM control antibody without anticoagulant activity and 11 lupus anticoagulant antibodies, were preincubated with DPPE (131 nmol), egg PE(PC) (100 nmol), bovine PE (5.4–13.5 nmol), egg PE (5.4–13.5 nmol), and dioleoyl-PE (*DOPE*, 2.7–67.5 nmol) at 37 °C, and then tested in the routine PTT assay. Each *bar* represents the mean PTT of duplicate samples. Antibody 1500 had no anticoagulant activity, and its partial thromboplastin time was not affected by the presence of any of the phospholipids. Incubation of the 11 hybridoma lupus anticoagulants with lamellar phospholipids, such as DPPE and egg PE(PC), resulted in PTT values equivalent to the PTT values of the antibodies plus buffer and, therefore, represent maximum PTT values for each antibody. In contrast, incubation of the 11 lupus anticoagulant antibodies with hexagonal phospholipids (bovine PE, egg PE, and dioleoyl-PE) caused a significant decrease ($p < 0.0005$) in the PTT to values equivalent to the 1500 IgM control.

binding activity of this antibody was achieved with the addition of 10 nmol of phosphorus of dDNA, while equivalent and 10-fold higher amounts of dioleoyl-PE and DPPE did not show any inhibition of the DNA-binding activity.

Similarly, the rheumatoid factor activity of hybridoma antibody 18141 (Fig. 3*B*) was inhibited by 70% with the addition of 10 μg of human IgG, but was unaffected by incubation with up to 100 nmol of phosphorus of dDNA, dioleoyl-PE, or DPPE.

Effects of Phospholipid Phase Structure on Lupus Anticoagulant Activity—Fig. 4 shows the effects of different PE phospholipid phase structures on the PTT of all 11 hybridoma lupus anticoagulant antibodies, as well as the 1500 IgM control. Hybridoma lupus anticoagulants incubated with buffer had PTT values ranging between 66 and 72 s. This represented a prolongation of 6–12 s over the IgM control, which had a mean PTT of 60 s. There was no correlation between anticoagulant activity and hybridoma IgM concentration, which ranged from 0.03–74.0 μg/ml.

Bovine and egg PE (which are hexagonal phase phospholipids at 37 °C) did not have any effect on the PTT assay itself, as their addition did not change the PTT of the control 1500 IgM supernatant. However, the addition of these phospholipids to the hybridoma lupus anticoagulants resulted in PTT values equivalent to the 1500 control for all 11 antibodies, demonstrating complete inhibition of lupus anticoagulant activity ($p < 0.0005$). Dioleoyl-PE (hexagonal phase at 37 °C) also completely inhibited the activity of all 11 hybridoma anticoagulants ($p < 0.0005$) but did not affect the control supernatant. Five of the 11 antibodies were inhibited by 2.7 nmol of dioleoyl-PE (one-half the amount of bovine PE added), while the other 6 required higher amounts of dioleoyl-PE (6.75–67.5 nmol of phosphorus) for complete inhibition. In contrast, DPPE and egg PE(PC) (both lamellar at 37 °C) caused no statistically significant inhibition of lupus anticoagulant activity at concentrations 1.5–48-fold that of dioleoyl-PE.

Effects of Preincubation of Hybridoma Lupus Anticoagulants with Egg PE(PC) at 37 and 43 °C—The previous results may be taken to suggest that the anticoagulant antibodies bind to hexagonal phase lipids as opposed to lipids in the lamellar organization. It is possible, however, that the observed effects may be due to the different molecular species of the phospholipids tested. In order to investigate this, we employed egg PE(PC), which adopts the lamellar phase at 37 °C and the hexagonal II phase at 43 °C (Fig. 1*B*). It was reasoned that if the antibodies are sensitive to structure, egg PE(PC) at 43 °C should be bound by the anticoagulant antibodies, whereas egg PE(PC) at 37 °C should not.

Egg PE(PC) and egg PE were incubated with the hybridoma anticoagulants at 43 °C and subsequently removed by Millipore filtration and the filtrate used in the PTT assay. Fig. 5 shows the effect of the preincubation of the 11 hybridoma lupus anticoagulants with egg PE(PC) at 37 and 43 °C and egg PE at 43 °C. Egg PE(PC) is lamellar at 37 °C and did not inhibit the lupus anticoagulant activity. However, egg PE(PC) assumes a hexagonal configuration at 43 °C (Fig. 1*B*) and, in this arrangement, dramatically inhibited the anticoagulant activity of the same lupus anticoagulants. In parallel experiments, egg PE was substituted for egg PE(PC) and it was found that at 43 °C, egg PE was less capable of inhibiting anticoagulant activity than at 37 °C, where it removed 100%

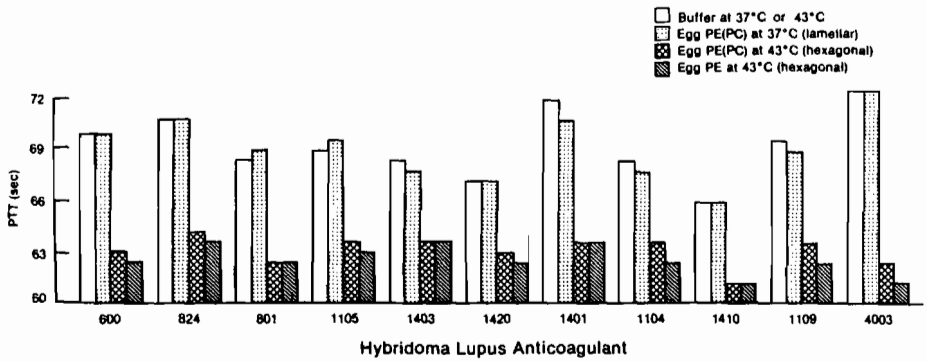

FIG. 5. **Effect of preincubation of hybridoma lupus anticoagulants with buffer and egg PE(PC) at 37 and 43 °C and egg PE at 43 °C.** Each *bar* represents the mean PTT of duplicate samples. The amounts of egg PE(PC) and egg PE added to the hybridoma antibodies were 89.8 and 114.4 nmol of phosphorus, respectively. Thus, egg PE(PC) in its hexagonal but not its lamellar conformation inhibited the lupus anticoagulant activity of all 11 anticoagulant antibodies.

of the lupus anticoagulant activity (Fig. 4). Thus, antibody binding to hexagonal lipid was found to be partially inhibited at elevated temperatures. Antibody heated to 43 °C in the presence of buffer retained all of its lupus anticoagulant activity.

DISCUSSION

The results of this study show that certain hybridoma antibodies derived from SLE patients can prolong normal PTT assay values and that this prolongation can be inhibited by the presence of hexagonal (H_{II}) but not lamellar phase phosphatidylethanolamine. Three points of interest concern the mechanism whereby these antibodies can increase clotting times, the reasons why they exhibit a preferential interaction with H_{II} phase phosphatidylethanolamines, and the possible biological significance of antibodies which interact with "non-bilayer" lipid structure. We discuss these points in turn.

The ability of the anticoagulant antibodies to prolong PTT values is not understood in detail. It is, however, well known that initiation of clotting requires the exposure of "inner monolayer" lipids such as PE and the negatively charged lipid phosphatidylserine on the outer monolayer of platelets (18). A similar surface is presumably provided by the cephalin dispersion in the *in vitro* PTT assay, and it may be that the presence of the anticoagulant antibodies impedes the binding of blood-clotting factors, thus prolonging the assay time. The ability of hexagonal phase (but not bilayer phase) PE to inhibit this prolongation clearly suggests a specific affinity of these anticoagulants for H_{II} phase lipid. Other hybridoma IgM autoantibodies with anti-DNA and rheumatoid factor activities cannot be inhibited by preincubation with hexagonal (H_{II}) phosphatidylethanolamine. Thus, these results support the "specific" recognition and interaction of the hybridoma lupus anticoagulants with hexagonal phase PE.

With the exception of DPPE, all of the phospholipids examined were in the fluid (liquid crystalline) state at the experimental temperatures employed. Thus, the inability of DPPE to reduce PTT values could be related to the crystalline (gel state) nature of this lipid species at 37 °C (19). However, the fact that egg PE(PC) was also incapable of inhibiting anticoagulant activity at 37 °C, where it exists in a liquid crystalline lamellar phase, clearly indicates that the overall polymorphic phase is the determining factor, rather than the gel or liquid crystalline nature of the acyl chains. The inability of phosphorylethanolamine and monomyristoyl or monopalmitoyl phosphatidylethanolamine to affect prolongation of the PTT values would also suggest that the anticoagulant-PE interaction does not solely involve a chemical interaction at the headgroup level. A remaining possibility concerns the nature of the interface between the H_{II} phase aggregates and the aqueous medium. In particular, it is logical to suggest that this interface consists of a monolayer of lipid which surrounds the aggregated hexagonally packed cylinders characteristic of the H_{II} phase (20). This monolayer would likely exhibit different packing properties than those observed in a bilayer system, possibly leading to increased antibody recognition and binding to epitopes unmasked on the ethanolamine portion of the molecule.

The results of this study indicate that SLE patients can exhibit antibodies which interact preferentially with, and are possibly specific for, H_{II} phase lipid structure. Current studies are aimed at demonstrating similar antibody specificity for non-bilayer lipidic particle structure (21). The possibility of antibodies against non-bilayer lipid aggregates may initially appear surprising. However, whereas the normal function of a cell membrane relies on the presence of a largely (if not exclusively) bilayer lipid organization, many membrane lipids preferentially adopt non-bilayer hexagonal phase or lipidic particle (21) structure. The long term presence of such structures *in vivo* could well represent lesions requiring recognition and clearance.

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