Hybridoma Lupus Anticoagulants Distinguish between Bilayer and Nonbilayer Phase Lipid Systems[a]

J. RAUCH,[b] M. TANNENBAUM,[b]
H. TANNENBAUM,[b] H. RAMELSON,[b] P. R. CULLIS,[c]
C. P. S. TILCOCK,[c] M. J. HOPE,[c] AND A. S. JANOFF[d]

[b]*Rheumatic Disease Unit*
Montreal General Hospital Research Institute
McGill University
Montreal, Quebec, Canada

[c]*Department of Biochemistry*
University of British Columbia
Vancouver, British Columbia, Canada

[d]*The Liposome Company*
Princeton, New Jersey

Antibodies to phospholipids represent a group of poorly characterized antibodies that may have important physiological and biological functions.[1] The mechanism(s) responsible for the production of these autoantibodies remains unknown. 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.[2] This possibility requires a clear demonstration that antiphospholipid antibodies exist that are able to distinguish different polymorphic forms of the same lipid.

Our approach to this problem has involved the use of human hybridoma lupus anticoagulant antibodies (LAA) derived from patients with SLE. LAAs are antiphospholipid antibodies[3] that are defined by their ability to prolong the normal clotting time in *in vitro* coagulation assays measuring the partial thromboplastin time (PTT). In this study, we show that the ability of LAA to prolong PTT values can be inhibited by the presence of hexagonal phase (but not bilayer phase) phosphatidylethanolamine (PE). This finding indicates an ability of these antibodies to distinguish between different structural configurations of the same chemical species of phospholipid.

METHODS

Human hybridoma LAAs were produced by fusing peripheral blood lymphocytes from SLE patients with the GM 4672 lymphoblastoid line.[4] The effects of different phospholipid systems on the partial thromboplastin times of 11 LAAs were assessed in

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the PTT assay following preincubation of the LAAs with phospholipids for ten minutes at 37°C. The polymorphic phase preferences of the phospholipids were determined by employing the ^{31}P NMR technique.[2]

RESULTS

Eleven of the 68 hybridoma IgM antibodies analyzed showed LAA activity. FIGURE 1 shows a titration curve of the inhibition of LAA activity of hybridoma

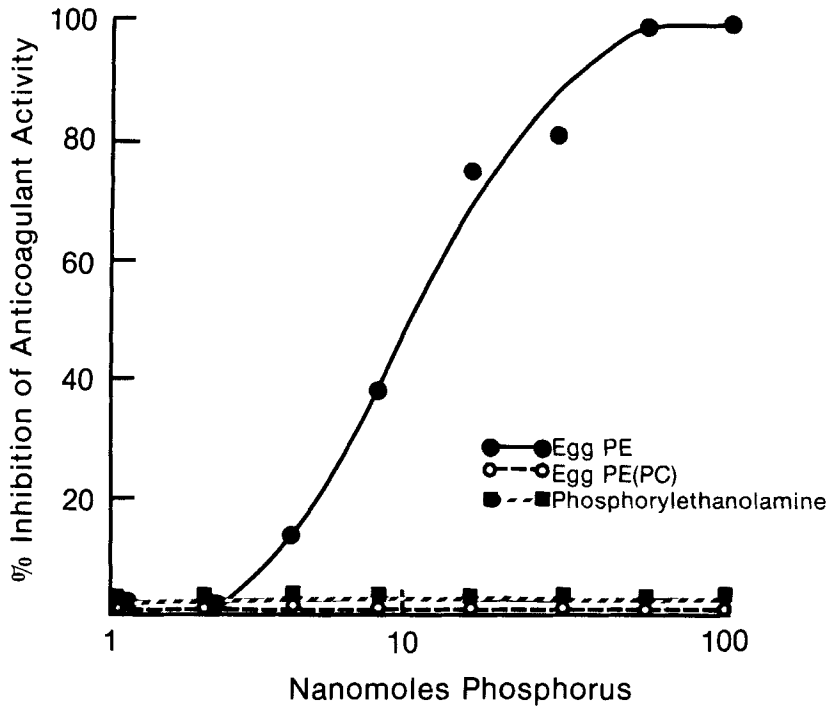

FIGURE 1. Titration of the inhibition of the anticoagulant activity of antibody 824 after preincubation with egg PE, egg PE(PC), or phosphorylethanolamine at 37°C for ten minutes. At this temperature, egg PE is hexagonal and egg PE(PC) is lamellar.

antibody 824. Complete inhibition of anticoagulant activity was obtained with the addition of egg PE (nonbilayer at 37°C) at 68 nmoles phosphorus, while no inhibition occurred with egg phosphatidylethanolamine derived from phosphatidylcholine [PE(PC)] (bilayer at 37°C) and phosphorylethanolamine, the polar-head group of PE.

The effects of different PE phospholipid phase structures on the PTT of 11 LAAs and a control IgM antibody (1500) are shown in FIGURE 2. Hybridoma LAAs incubated with buffer had PTT values ranging between 66–72 sec, representing a prolongation of 6–12 sec over the IgM control (PTT = 60 sec). Bovine PE, egg PE, and

dioleoylphosphatidylethanolamine (DOPE) (which are all hexagonal at 37°C) did not affect the PTT of the control 1500 IgM, but reduced the PTT values of the 11 hybridoma LAAs to the control value ($p < 0.0005$), thereby showing complete inhibition of lupus anticoagulant activity. In contrast, dipalmitoylphosphatidylethanolamine (DPPE) and egg PE(PC) (which are both lamellar at 37°C) caused no inhibition of LAA activity at concentrations 1.5–48-fold that of DOPE.

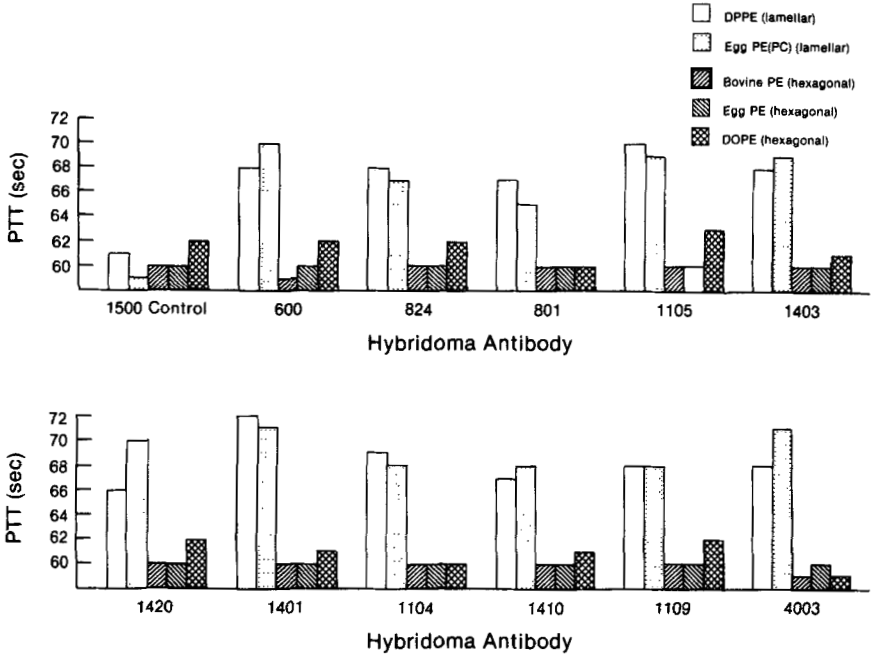

FIGURE 2. Effects of preincubation of different phospholipid phase systems with hybridoma lupus anticoagulants at 37°C. Incubation of the 11 hybridoma lupus anticoagulants with lamellar phospholipids [such as DPPE and egg PE(PC)] resulted in PTT values equivalent to the PTT values of the antibodies plus buffer. They, therefore, represent the maximum PTT values for each antibody. In contrast, incubation of the 11 lupus anticoagulant antibodies with hexagonal phospholipids (bovine PE, egg PE, and DOPE) caused a significant decrease ($p < 0.0005$) in the PTT to values equivalent to the 1500 IgM control.

DISCUSSION

Our results indicate that LAAs react preferentially with hexagonal phase lipid structures. The possibility of antibodies against nonbilayer lipid structures 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 nonbilayer hexagonal phase or lipidic particle[5] structure. The long-term presence of such structures *in vivo* could well represent lesions requiring antibody recognition and clearance.

REFERENCES

1. ALVING, C. R. 1984. Biochem. Soc. Trans. **12:** 342.
2. CULLIS, P. R. & B. DE KRUIJFF. 1979. Biochim. Biophys. Acta **559:** 399.
3. THIAGARAJAN, P., S. S. SHAPIRO *et al.* 1980. J. Clin. Invest. **66:** 397.
4. MASSICOTTE, H., J. RAUCH *et al.* 1984. Hybridoma **3:** 215.
5. VERKLEIJ, A. J., C. MOMBERS *et al.* 1979. Nature **279:** 162.