PHOSPHATIDYL CHOLINE IS RECOGNIZED BY A SERIES OF Ly-1$^+$ MURINE B CELL LYMPHOMAS SPECIFIC FOR ERYTHROCYTE MEMBRANES

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A minor population of murine B lymphocytes express the cell surface antigen Ly-1 (1), and a similarly small proportion of human B cells carry the homologous antigen Leu-1/T1 (2). In normal mice, ~2–5% of splenic B cells bear Ly-1, but these include virtually all of the cells that spontaneously secrete antibody hemolytic for SRBC or for autologous erythrocytes treated with the proteolytic enzyme bromelain (BrMRBC).[1] In NZB mice, which develop congenital autoimmune disease, the frequency of Ly-1$^+$ B cells is elevated (3), and they are reportedly responsible for the production of all the anti-DNA antibodies associated with the disease (4).

We have previously described (5) 27 independently derived murine B cell lymphomas in the CH series. At least 70% of these tumors bear Ly-1, their Ig are idiotypically related one to another, and several recognize SRBC and BrMRBC via their surface Ig (5). The spleens of normal syngeneic mice contain Ly-1$^+$ B cells that produce antibody hemolytic for SRBC and BrMRBC. About half of these cells are idiotypically crossreactive with the erythrocyte-reactive CH lymphomas (6). This implies that the CH lymphomas represent neoplastic analogs of the Ly-1$^+$ normal B cells.

During an experiment using antibody-conjugated, dye-loaded liposomes as a second-step immunofluorescence reagent, we noted that CH12 tumor cells bound them independently of the addition of first-step antibody. Binding was not a function of the specificity of the antibody conjugated to the liposomes, but occurred with all liposomes of similar phospholipid composition. Given that the liposomes are constructed with phospholipids similar to those present in erythrocyte membranes and that some erythrocytes bind to members of the CH series by way of surface IgM (sIgM), we decided to explore the possibility that the particular epitope recognized by CH12 Ig was included as a component of the fluorescent dye–loaded liposomes.

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[1] *Abbreviations used in this paper:* B-CLL, B cell chronic lymphocytic leukemia; BrMRBC, bromelain-treated mouse red blood cells; DSPC, distearoylphosphatidyl choline; DSPG, distearoylphosphatidyl glycerol; GARb, goat antiserum to rabbit Ig.

After showing that liposome attachment to CH12 cells was due to binding to sIgM, we tested a panel of other CH tumor cells for their ability to bind liposomes. We report here that all six of the CH lymphomas that bear sIgM reactive with both SRBC and BrMRBC also bind our tracer liposomes. Experiments involving blocking of liposome binding on the cell surface and a competition immunoassay have enabled us to show that the epitope recognized by these tumors is the phosphatidyl choline moiety of membrane phospholipids. We also discuss significance of this finding in relation to other commonly studied autoantigens and to the restricted specificity of normal Ly-1$^+$ B cells.

Materials and Methods

Mice and Tumors. B10.*H-2^{a}H-4^b*p/Wts (2^a4^b) were bred and maintained in our pathogen-free mouse colony. This strain had been derived from selected F_2 progeny of B10.A × B10.129(21M) mice (7). The CH series of B cell lymphomas arose in 2^a4^b mice following adoptive hyperimmunization with SRBC. Details concerning the induction of CH1-CH12 have been reported by Lanier et al. (8), and Pennell et al. (5) have reported on CH15-CH39. The tumors were maintained by serial transplantation of ascites or splenic tissue in syngeneic animals, or in tissue culture in RPMI 1640 supplemented with 10% FCS, glutamine, gentamycin and 2-ME.

Liposomes. Fluorescein-encapsulating, Ig-conjugated liposomes were prepared by a modification of the reverse-phase evaporation method of Szoka and Papahadjapoulos (9). Briefly, 119 μmoles distearoylphosphatidyl choline (DSPC) (Avanti Polar Lipids, Birmingham, AL), 13.2 μmoles distearoylphosphatidyl glycerol (DSPG) (Avanti Polar Lipids), 3.75 μmoles of a maleimido derivative of distearoyl phosphatidyl ethanolamine (gift of Dr J. P. O'Connell, Becton Dickinson Research Center, Research Triangle Park, NC) and 132 μmoles of cholesterol (Sigma Chemical Co., St. Louis, MO) were dissolved in 6:1 chloroform/methanol. This lipid solution was emulsified by sonication with 2/7 volume of 5 mM 6-carboxyfluorescein (Eastman Kodak, Rochester, NY) in an isotonic Tris-EDTA buffer, pH 5.5. Organic solvent was then removed by rotary evaporation. This preparation was warmed above the transition temperature, then extruded through 0.4- and 0.2-μm filters in the presence of an additional five volumes of dye solution. Unencapsulated dye was washed away by ultracentrifugation. Affinity-purified goat anti-rabbit Ig, heavy and light chain-specific (Cappel Laboratories, Cochranville, MD) or human IgG (Miles Pentex, Los Angeles, CA) were reduced with DTT. Reduced antibodies were allowed to react with prepared liposomes overnight at room temperature. Unconjugated protein was washed away by ultracentrifugation.

Liposomes used in the solid-phase IgM-mediated binding assay were the kind gift of Dan McLaurin (Becton-Dickinson Research Center). Their lipid formulation was identical with that of the carboxyfluorescein liposomes described above. These vesicles encapsulated sulforhodamine-B and had no protein conjugated. Stock suspensions of both dye-loaded liposomes were adjusted to ~2 mM in inorganic phosphate as determined by the method of Fiske and SubbaRow (10).

Empty vesicles used in CH-lymphoma blocking experiments were prepared by drying 34 μmoles DSPC or DSPG plus 33 μmoles cholesterol to a thin film on a round-bottom flask. Lipids were then dispersed in PBS above the transition temperature and extruded through a 0.2 μm filter.

Liposome Binding to CH-Lymphoma Cells. An equal volume of cell suspension, containing 10^7 viable cells/ml in HBSS plus 0.1% BSA was incubated with a 1:10 dilution of empty DSPC- or DSPG-vesicles, or medium only, for 1 h at 37°C (capping conditions), or for 20 min on ice in the presence of NaN_3 (blocking conditions). Cells were washed twice with HBSS/BSA/NaN_3, then resuspended to the original volume. Aliquots (100 μl) of tumor cells were then incubated with 25 μl of rabbit anti-mouse μ-chain antibody (Litton Bionetics, Kensington, MD) at a 1:4 dilution, for 30 min on ice. Controls were 1:4 dilutions of normal rabbit serum or buffer alone. Three washes with HBSS followed. Liposomes

conjugated with goat anti–rabbit Ig were added (50 μl of a 1:10 dilution) and incubated on ice for 30 min. Cells were then washed three times with PBS plus NaN_3. Analysis was performed on an EPICS V cell sorter (Coulter Electronics, Hialeah, FL) using the 488 nm line from an argon-ion laser. Cells that did not bind liposomes directly were examined for indirect binding mediated by the rabbit anti-μ antibody and the anti–rabbit Ig specificity of the liposomes.

Quantitation of Cell Surface IgM. Surface IgM of CH-lymphomas was determined by indirect immunofluorescence. 100 μl of tumor cells at 10^7 cells/ml were incubated for 20 min on ice with rat mAb specific for mouse IgM (clone 331.12, [11]) or no antibody as control, washed twice with HBSS/NaN_3, then stained with fluoresceinated mouse anti–rat κ chain mAb (MAR18.5, Becton Dickinson Immunocytometry Systems, Mountain View, CA). After three washes with PBS/NaN_3, cells were resuspended to 10^6 cells/ml and analyzed on the EPICS V as described above.

Liposome Agglutination. A hybridoma was prepared by fusing CH12 lymphoma cells with the P3-X63-Ag8.653 nonsecreting myeloma cell line as described (5). Agglutination was performed by using 15 μl of supernatants from CH12 hybridoma cells, or 25 μl of a hybridoma supernatant with an irrelevant antigen specificity (H013.4, anti-Thy-1.2, [12]) grown in the same medium. 25 μl of human IgG–conjugated, fluorescein-containing liposomes were then mixed with the antibodies on a glass slide with a black background. After 60 min at room temprature, results were visualized using a hand-held ultraviolet lamp.

IgM-mediated Liposome Binding to Polyacrylamide Beads. One vial of goat anti–rabbit Ig polyacrylamide beads (Immunobead Second Antibody; Bio-Rad Laboratories, Richmond, CA) at 4 mg/ml in 150 mM EDTA, pH 7.5, with 0.1% dextran (assay buffer) was reacted for 5 min at 37°C with 16 ml of a 1:50 dilution of affinity-purified rabbit anti–mouse μ chain antibody (Jackson Immunoresearch Laboratories, Bar Harbor, ME), then washed three times with assay buffer. The pellet was then resuspended to the original 50-ml volume, and 25 ml reacted with 8 ml of a 1:10 dilution of the IgM-containing supernatant of the CH12 hybridoma (concentrated 10-fold by addition of saturated ammonium sulfate concentrate). An additional 10 ml of anti–mouse μ-specific beads was reacted with 3.2 ml of a 1:10 dilution of CH10 hybridoma supernatant. After 5 min at 37°C, both preparations were washed three times with assay buffer.

Blocking of the IgM-mediated liposome binding to the polyacrylamide beads was then attempted. Inhibitor phospholipid suspensions were prepared by drying under a stream of nitrogen, 1 ml of a 20 mg/ml solution of DSPC (Sigma Chemical Co.), sphingomyelin (Sigma Chemical Co.), or DSPG (Avanti Polar Lipids) in chloroform to the wall of a clean glass scintillation vial. The dry lipid film was then dispersed in 10 ml of 150 mM EDTA, pH 7.5, at 57°C, and extruded at this temperature through a 0.22-μm Millex GV filter prewashed with the buffer.

750 μl of the incubators, at 0.5–0.0625 mg/ml input phospholipid, were incubated with 500 μl CH-IgM bead suspension for 15 min on ice, then 750 μl of a 1:80 dilution of the stock suspension of sulforhodamine-B liposomes was added, and the incubation was continued for an additional 5 min. 3 ml of PBS was then added, and the tubes were centrifuged. Solid-phase bound liposomes were lysed with 1% Triton X-100, and the tubes were recentrifuged. Absorbance of the supernatant at 565 nm was then measured.

Results

Liposome Binding. Cells from 14 different CH lymphomas were tested for binding of the fluorescent liposomes described above. Six of the tumors bear surface IgM with specificity for SRBC and BrMRBC, and one of these (CH34) also binds *E. coli*. Two tumors bear IgM that recognizes only BrMRBC, while the Ig expressed by two others recognize only *E. coli* (4). Data are presented in Table I. Liposomes were bound directly by all six tumors with the SRBC and BrMRBC specificity, but not by any of the others. Failure of a tumor to bind

TABLE I

Direct Binding of Fluorescent Liposomes and Its Inhibition by Pure Compounds

Cells	Antigens bound*	Binding of liposomes (peak fluorescence channel)		
		Alone	DSPC	DSPG‡
CH1	E	<1	—	—
CH6	—	<1	—	—
CH9	B	<1	—	—
CH10	E	<1	—	—
CH12	S, B	150	82	150
CH15	B	<1	—	—
CH27	S, B	188	<1	184
CH28	S, B	150	31	150
CH29	—	<1	—	—
CH30	—	<1	—	—
CH32	S, B	144	<1	149
CH34	S, B, E	211	140	200
CH35	S, B	125	<1	120
CH39	—	<1	—	—

* Antigen-binding specificity of sIgM. E, *E. coli* cells; B, bromelain-treated mouse erythrocytes; S, sheep erythrocytes (from Pennell et al. [5]).

‡ Cells were stained with fluorescent dye–loaded liposomes with DSPC, DSPG, and cholesterol as membrane components. Inhibitors were colorless vesicles of DSPC/cholesterol or DSPG/cholesterol. Data are the peak fluorescence channel numbers determined by flow cytometry using a 256-channel, three-decade logarithmic scale; <1, fluorescence peak not detected on-scale; —, test not applicable.

liposomes directly was not due to absence of sIgM, since liposomes conjugated with goat anti–rabbit Ig (GARb) would bind indirectly after preincubation of tumor cells with rabbit anti–mouse IgM sera. We took these data to imply that the determinant on SRBC and BrMRBC recognized by CH12, -27, -28, -32, -34, and -35 was the same as, or similar to some surface component of our synthetic liposomes. As a corollary, the surface component of BrMRBC recognized by CH9 and -15 must differ from the above epitope and be absent from the liposomes.

We undertook blocking experiments to determine which of the liposome components carried the epitope recognized by the six SRBC-specific tumors. Empty vesicles were prepared with lipid compositions of 50 mol-percents DSPC plus 50 mol-percents cholesterol, or 50:50 DSPG/cholesterol. Preincubation of tumor cells with DSPC- but not DSPG-containing vesicles reduced the binding of fluorescent liposomes from 10-fold (partial blocking) to >1,000-fold (complete blocking). The relative degree of blocking was the same whether conducted under capping or noncapping conditions. Table I also gives the fluorescence intensity observed for the liposome-binding CH lymphomas, and results of blocking experiments. Fig. 1 contains sample fluorescence histograms from flow cytometric analysis, which show direct binding of liposomes to CH-tumors, blocking by empty vesicles, and use of GARb-conjugated liposomes as second-step reagents for indirect staining of sIgM on CH39. Data for CH27 are

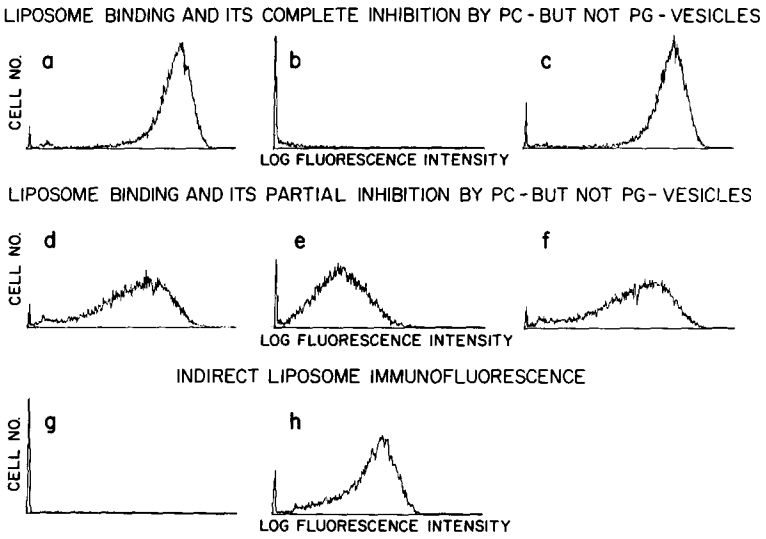

FIGURE 1. CH27 cells preincubated with RPMI alone (*a*), empty DSPC/cholesterol liposomes (*b*), or empty DSPG/cholesterol liposomes (*c*), 60 min, 37°C, followed by staining with DSPC/DSPG/cholesterol liposomes encapsulating carboxyfluorescein and conjugated with goat anti-rabbit Ig antibody. CH12 cells in *d*, *e*, and *f* treated as in *a*, *b*, and *c* above, respectively. CH39 cells (*g*) were preincubated as in *a* and *d* above, and serve as an example of failure to bind liposomes directly, and as a control for the indirect liposome immunofluorescence shown in *h*, where cells were stained with rabbit anti-mouse μ chain antibody, followed by fluorescent goat anti-rabbit Ig liposomes.

representative of high-density binding of fluorescent liposomes and complete blocking by preincubation with DSPC-conjugated but not with DSPG-conjugated vesicles. CH12 is an example of lower-density binding and only partial inhibition by DSPC. Note (Table I) that a high density of liposome binding can be associated with either complete or partial blocking by preincubation with DSPC vesicles; similarly, tumors that bind liposomes at low density are also blocked by DSPC-conjugated vesicles to different extents. Thus, the degree of inhibition is not a simple function of the number of liposomes bound, but is more likely related to the avidity of binding.

These data imply that the phosphatidylcholine moiety mediates liposome binding to the SRBC rosette-forming CH-lymphomas, and that binding avidity differs between tumors.

Specificity of CH12 IgM for Liposomes. As a direct demonstration that cell-surface Ig was responsible for binding liposomes, we tested the IgM secreted by a hybridoma made by fusing CH12 with the nonsecreting myeloma P3-X63 Ag 8.653. As a control, we used the culture supernatant from H013.4, an anti-Thy-1.2 murine IgM, grown under the same conditions. Dilutions of both IgM were incubated with suspensions of the liposomes used in the cell-labelling experiments. Agglutination occurred with CH12 Ig, but not with H013.4 hybridoma supernatant (data not shown).

Correlation of sIgM Density with Liposome Binding. Since the density of liposomes bound by cells of the various CH tumors differed over a 100-fold range, we wanted to know whether this was a function of cell surface concentration of

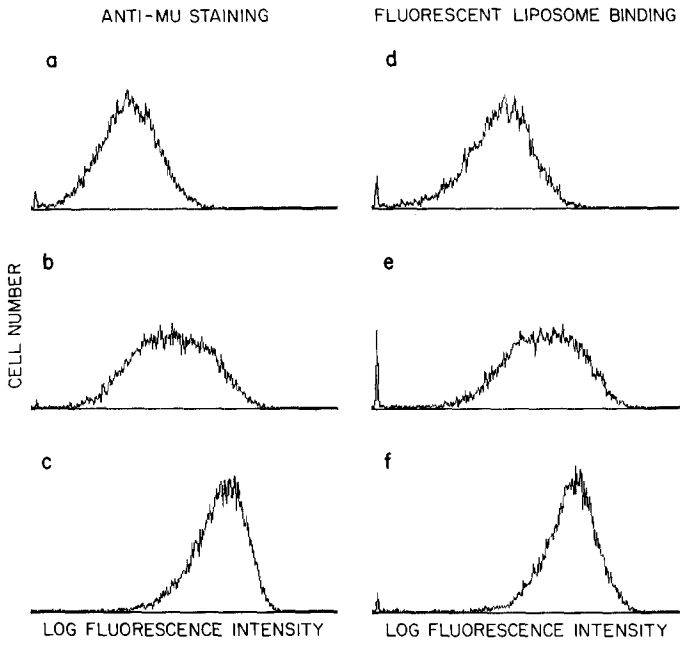

FIGURE 2. Fluorescence intensity histograms of CH12 (*a* and *d*), CH34 (*b* and *e*), and CH27 (*c* and *f*) lymphomas generated by staining cell populations for surface IgM (*a*, *b*, and *c*) or by direct binding of carboxyfluorescein-loaded liposomes (*d*, *e*, and *f*). Histograms (*a–c*) were collected at a higher sensitivity than *d–f* due to the lower fluorescence intensity of samples stained with fluoresceinated antibodies compared to those stained with dye-loaded liposomes; data collection using logarithmic amplifiers allows for comparison of intensity distributions.

IgM. Indirect immunofluorescence was used to measure the relative concentrations of sIgM, and this was compound to the density of liposomes bound. The log of the peak fluorescence for sIgM and for liposome binding were compared by linear regression analysis. A correlation coefficient (r^2) of >0.9 was obtained, indicating a major contribution of the cell-surface IgM concentration to determining the degree to which a particular CH lymphoma cell population binds the fluorescent liposomes.

Sample histograms from flow cytometry analysis for sIgM fluorescence intensity and for liposome binding are compared in Fig. 2. Shapes of the distribution curves for sIgM and for the direct binding of fluorescent liposomes are virtually indistinguishable. The sIgM concentration thus affects liposome binding intensity on a per-cell basis to the same degree as observed for the total population.

Solid-phase Competition Immunoassay. Polyacrylamide beads with CH12 IgM attached were used to measure inhibition of the CH12/DPSC interaction by pure compounds. Beads bearing CH10 IgM served as controls. Addition of DSPC/DSPG/cholesterol liposomes loaded with sulforhodamine B (~25 nmol inorganic phosphate, 0.717 absorbance units at 565 nm) led to 55.5% liposome binding to CH12 solid-phase in 5 min. The CH10 solid phase bound <10% of the total encapsulated dye.

This absorbance assay was then used to test inhibition of the CH12/liposome interaction by pure compounds. Binding of the dye-loaded tracer vesicles could

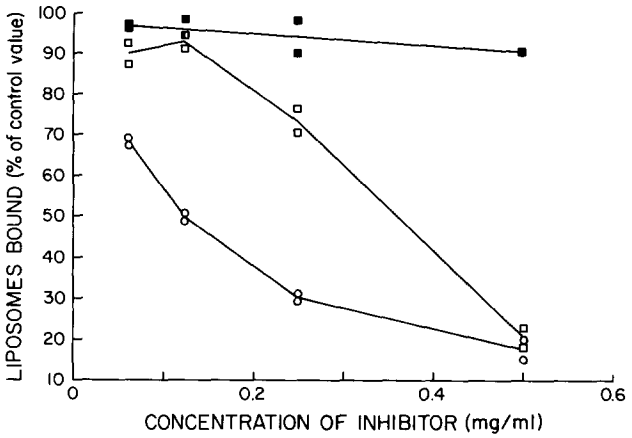

FIGURE 3. Inhibition of binding of sulforhodamine B–loaded liposomes to solid-phase CH12 IgM. Polyacrylamide beads coated with CH12 IgM were incubated with liposomes in the presence of the indicated concentrations of inhibitors. (□), DSPC; (○), sphingomyelin; (■), DSPG. Liposome binding was determined spectrophotometrically, and is expressed as the percentage of the value observed in the absence of any added inhibitor.

be inhibited specifically by addition of aqueous dispersions of either DSPC or sphingomyelin (which share only the phosphatidylcholine moiety), but not by DSPG. Competition curves were generated and are shown in Fig. 3. The apparently higher avidity of the CH12 IgM for sphingomyelin than for DSPC may be due either to vesicle structure (sphingomyelin may form fewer multilammelar vesicles and hence present greater membrane surface per unit weight), or to alternative conformation of the polar head groups in the two compounds. In any event, it is clear that the phosphatidyl choline moiety is the epitope recognized by CH12.

Discussion

Six of the CH series of B cell lymphomas bear surface Ig that recognize determinants on both SRBC and BrMRBC (3). We have shown here that the CH lymphomas with this erythrocyte specificity also recognize the phosphatidyl choline moiety in synthetic liposome membranes. In addition to these six tumors, the CH series also includes five lymphomas whose cells form rosettes with *E. coli*, but not with erythrocytes, and three that react with BrMRBC but not with SRBC (5). Although none of these eight tumors binds our indicator liposomes, their surface Ig are idiotypically related to those that do. Furthermore, one of the tumors that has now been shown to bind phosphatidyl choline (CH34) also crossreacts with *E. coli*. These data imply that there is a structural similarity between the various epitopes recognized by this group of B cells.

We have noted that, although all SRBC/phosphatidyl choline–specific tumors also bind BrMRBC, there are three CH lymphomas with specificity for BrMRBC only, and these tumors do not bind DSPC-containing liposomes. This implies that BrMRBC carry two different cell surface epitopes that are recognized by different tumors, but which might be expected to be chemically related structures.

Based on the observation that the CH lymphomas display a restricted repertoire of idiotopes and antigen-binding specificities, Pennell et al. (5), concluded that these tumors derived from a subset of B cells defined by the expression of a limited number of V_H gene products. Bishop and Haughton (6) found that the idiotype of one of these tumors, CH12, is also expressed by a high proportion of normal 2^a4^b spleen cells, which form spontaneous plaques with both SRBC and BrMRBC. We have now shown that the Ig of CH12 and five other lymphomas with specificity for SRBC and BrMRBC recognize the phosphatidyl choline epitope. Although less than formal proof, these observations strongly suggest that binding of the Ig to erythrocytes involves the phosphatidyl choline groups associated with membrane lipids. We propose that this is the basis for spontaneous plaque formation by normal splenic B cells. Further substantiation is also given to the conclusion that the CH lymphomas are derived from a subset of B cells, the normal counterpart of which includes the spontaneous plaque-forming cells.

Lord and Dutton (13) suggested that the spontaneous plaque-forming cells found in normal spleens are representative of an autoimmune response against antigenic determinants to which the animal is continuously exposed. They suggested that the determinant recognized on BrMRBC becomes exposed in vivo on effete erythrocytes, providing a means for their recognition and elimination. More recently, Grabar (14) has suggested that antibodies' protective role as defenders against microbial invasion may have evolved from a more ancient physiological function as transport molecules and in internal homeostasis. Based on the high incidence of naturally occurring antibodies to liposomes and phospholipids in normal human sera, Alving (15) also proposed a physiological function for these autoantibodies. Hayakawa et al. (16), have recently shown that Ly-1$^+$ B cells have a lineage distinct from the majority of other B cells. This group had previously proposed that Ly-1$^+$ B cells are responsible for the production of several commonly studied IgM autoantibodies (4). They showed that FACS-sorted IgM$^+$, Ly-1$^+$ cells from young NZB mice synthesized essentially all of the IgM anti-DNA antibodies seen in in vitro splenic cultures from these animals. FACS-sorted Ly-1$^+$ B cells from BALB/c mice contained the subpopulation which, upon LPS stimulation, formed hemolytic plaques with BrMRBC. Given that the SRBC/DSPC-specific CH lymphomas all express the Ly-1 surface marker (5), our data suggest a relationship between the spontaneous antierythrocyte and anti-DNA specificities. Precedent for such a claim can be inferred from a recent report from Diamond and Scharff (17). From cultures of the phosphoryl choline–binding myeloma S107, a variant was selected that makes an Ig with only a single amino acid difference from the parental line, resulting in a loss of phosphoryl choline binding and an acquired reactivity with DNA.

There are also several well-known antibody crossreactivities resulting from the similarity in chemical structure between phospholipid membranes and the repeating phosphodiester linkages found in nucleic acids. The classic example of such crossreactivity is the false VDRL-positives due to the recognition of cardiolipin by anti-DNA antibodies present in the sera of SLE patients (18). Some SLE sera are also known to have anticoagulant activity residing in the Ig fraction, which is crossreactive with antiphospholipid and anti-DNA antibodies (19). Lafer et al. (28), produced hybridomas from spleens of unimmunized MRL/1 mice

(which spontaneously develop severe SLE). Using competitive RIA, they were able to demonstrate that a variety of phospholipids would block binding of hybridoma antibodies to denatured DNA. One antibody prolonged the partial thromboplastin time, as is characteristic of lupus anticoagulant, and this activity could be blocked by cardiolipin. They concluded that a range of abnormalities seen in SLE could result from binding of certain autoantibodies to biologically ubiquitous phosphodiester-containing epitopes.

In humans, tumors of B cell chronic lymphocytic leukemias (B-CLL) present phenotypic characteristics (reviewed in 21) that are strikingly similar to those of the CH series of murine lymphomas. Typical of B-CLL is the expression of the Leu-1/T1 surface marker, the human homolog of Ly-1 (2). In concordance with this observation is the frequent preceding or concomitant manifestation of autoimmune phenomena in association with B-CLL. Most notably, ~20% of B-CLL patients develop autoimmune hemolytic anemia (21), consistent with an antiphospholipid specificity of the mAb produced by the tumor. We are aware of at least one reported case of a B-CLL producing an IgM crossreactive with cardiolipin, phosphatidyl choline and several other phospholipids (22). Other human B-CLL are likely to share this antigen specificity; rosettes with SRBC have been reported to be formed by leukemic B cells via their cell surface Ig (17). The normal Leu-1/T1$^+$ B cell precursors of B-CLL may play a role in other autoimmune diseases, as has been suggested for the murine Ly-1$^+$ subset by Hayakawa et al., (4). Caligaris-Cappio and Janossy (21) recently proposed such a role for these Leu-1/T1 cells in human SLE. The question remains to be answered whether reactivity for phosphorylated autoantigens is characteristic of malignant B cells, or, as seems more likely, is a feature shared by normal and aberrant Ly-1$^+$ (Leu-1/T1$^+$) B cells. The use of fluorescent liposomes promises to be a powerful technique for addressing this question.

Summary

Cells from 6 of 14 different Ly-1$^+$ murine B cell lymphomas bound to synthetic liposomes encapsulating fluorescein. The liposomes were made from distearoyl phosphatidyl choline (DSPC), distearoyl phosphatidyl glycerol (DSPG), and cholesterol. In all cases, liposome binding was due to recognition of phosphatidyl choline by the surface IgM on the tumor cells. Liposome binding could be inhibited by DSPC but not by DSPG, and the number of liposomes bound per cell was directly related to the cell surface concentration of IgM. The IgM secreted by a hybridoma derived from one of the lymphomas, CH12, was shown to agglutinate liposomes, and was used in a solid-phase immunoassay to study inhibition of liposome binding by pure phospholipids; DSPC and sphingomyelin both inhibited, whereas DSPG did not.

The Ig borne by the six lymphomas that bind phosphatidylcholine also bind to both SRBC and bromelain-treated mouse erythrocytes. The idiotypic of CH12 IgM is similar to that expressed by Ly-1$^+$ normal splenic B cells of the same specificity. The significance of these data in relation to other commonly studied autoantigens, and to the restricted specificity of normal Ly-1$^+$ B cells is discussed.

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