NORMAL MOUSE PERITONEUM CONTAINS A LARGE POPULATION OF Ly-1$^+$ (CD5) B CELLS THAT RECOGNIZE PHOSPHATIDYL CHOLINE

Relationship to Cells that Secrete Hemolytic Antibody Specific for Autologous Erythrocytes

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The most remarkable feature of the immune response is its ability to generate cells and antibodies specifically reactive with an enormous variety of foreign antigens while normally avoiding the production of autoreactive elements. An accumulating body of data demonstrates that a distinct lineage of B cells (1), the Ly-1$^+$ B cell subset, does not display this feature, but rather is associated with the production of autoreactive antibodies (2). We are unaware of autoimmune pathology directly demonstrated to be the result of an Ly-1$^+$ B cell–Ig product; however, Ly-1$^+$ B cell frequency is elevated in some autoimmune strains of mice (3). In addition to their association with autoreactive antibody, Ly-1$^+$ B cells have been shown to have unique growth potential (1, 4); to produce most of the serum IgM found in normal, unimmunized animals (4); and the Ig repertoire of the subset may be restricted (4, 5). These findings imply that this subset has a role in normal physiology, and perhaps autoimmune pathology. A B cell subset has been identified in humans that bears the homologous marker Leu-1/T1 (CD5) (6). A similar functional role for CD5$^+$ B cells in man has been proposed (7); human CD5$^+$ B cells have been shown to produce autoreactive antibody (8, 9) and to be present at elevated frequency in rheumatoid arthritis (10).

Normal mice produce antibodies that are reactive with protease-treated autologous erythrocytes (BrMRBC)[1] (11); cells producing such antibodies are present in unimmunized mice at very high frequencies, as first demonstrated by Cunningham (12). We and others (13–15) have shown that at least some anti-BrMRBC plaque-forming cells (PFC) recognize the polar headgroup of the common membrane phospholipid, phosphatidyl choline (PtC). Most splenic anti-BrMRBC PFC have been

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[1] *Abbreviations used in this paper:* BrMRBC, mouse erythrocytes treated with the proteolytic enzyme bromelain; CF, 6-carboxyfluorescein; PerC, peritoneal cells; PFC, plaque-forming cells; PtC, phosphatidyl choline; SRB, sulforhodamine B.

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shown to bear the cell surface marker, Ly-1 (2, 16), raising the possibility that anti-PtC is contained within the putatively restricted repertoire of Ly-1+ B cells.

We demonstrate here that, in the peritoneums of normal adult mice, a large number of lymphocytes bind a fluorescent liposome probe. We show that these cells bind the liposomes to cell surface IgM by recognizing PtC. Virtually all of these PtC-specific cells bear the cell surface marker Ly-1. We have found that all the cells that form plaques on BrMRBC are included in the PtC-specific population and can be isolated by FACS. We believe this is the first report of sorting large numbers of B cells with a single antigen specificity from normal, unimmunized animals. This method will allow for in vitro and in vivo studies of differentiative and proliferative properties of Ly-1+ B cells, which may help define their role in development and disease.

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Materials and Methods

Mice. B10.H-2a-H4^{b}p/Wts (2^{a}4^b) mice were bred and maintained in our pathogen-free mouse colony. This strain had been developed from selected F_2 progeny of B10.A × B10.129 (21M) mice (17). Dr. Charles Sidman (The Jackson Laboratories, Bar Harbor, ME) kindly provided breeding stock of C3H mice heterozygous for the motheaten viable (*me^v*) allele (18). These mice were also bred in our mouse colony.

Liposomes. Fluorescent dye-entrapping liposomes were prepared as previously described (14), except no disulfide-reactive phospholipid was included in the formulation and no antibody was coupled to the liposome surface. Liposomes were composed of 45-mole percent synthetic distearoylphosphatidyl choline, 5-mole percent distearoylphosphatidyl glycerol (Avanti Polar Lipids, Birmingham, AL), and 50-mole percent cholesterol (Sigma Chemical Co., St. Louis, MO) and entrapped either 5 mM 6-carboxyfluorescein (CF) or sulforhodamine-B (SRB) (Eastman Kodak Co., Rochester, NY). Extrusions through microporous membranes produced liposomes of <0.2 μm in diameter.

Preparation of Cell Suspensions. Mice were killed by cervical dislocation (normal adults) or CO_2 asphyxiation (motheaten mice and mice <3 wk old). Peritoneal cells (PerC) were obtained by injecting 5–10 ml RPMI 1640, supplemented with 10% FCS, glutamine, 2-ME, and gentamicin (B cell medium), into the peritoneal cavity, gently agitating, and withdrawing medium aseptically with a Pasteur pipette. Cells were washed once and adjusted to 5×10^6/ml with the same medium. Samples that contained contaminating erythrocytes were discarded. Spleen, lymph node, and neonatal liver cell suspensions were prepared by teasing these organs between frosted glass slides. Erythrocytes were removed from spleen and neonatal liver samples by treating them with Gey's solution.

Immunofluorescence Analysis. 50 μl of CF liposomes at ~2 mM inorganic phophate were added per 5×10^5 cells. This mixture was incubated for 20 min on ice then washed twice. For indirect immunofluorescence staining, 5×10^5 cells were first incubated with saturating amounts of rat anti-Ly-1 (clone 53-7.3, reference 19), anti-Ly-2 (clone 53-6.7, reference 19), anti-Thy-1 (clone HO13.4, reference 20), anti-IgM (clone 331.12, reference 21), or medium only for 20 min, washed twice, then reacted with mouse anti-rat κ fluorescein conjugate (MAR 18.5-FITC; Becton Dickinson & Co., Mountain View, CA). CF liposomes, SRB liposomes, or Texas Red–conjugated goat anti-mouse IgM (Fisher Biotech, Orangeburg, NY) were used as direct immunofluorescence reagents. Flow cytometric analysis and sorting was performed using an EPICS V cell sorter (Coulter Electronics, Hialeah, FL) with 488 nm excitation from an argon-ion laser and 568 nm excitation from a krypton-ion laser. We used PBS, which contained no preservative, as the sheath fluid for all sorting experiments.

Assay for Anti-BrMRBC PFC. Sorted peritoneal lymphocytes were washed and resuspended to 4×10^4 or 3×10^5 cells/ml in RPMI 1640 supplemented with 10% heat-inactivated FCS, 300 μg/ml fresh glutamine, 10^{-5} M 2-ME 50 μg/ml LPS W (Difco Laboratories, Inc., Detroit, MI), and antibiotics. Cultures were performed by incubating 200 μl of cell suspension per well of a 96-well tissue culture plate at 37°C in a 5% CO_2 atmosphere for 2–4 d.

After culture cells were harvested and viable cells were counted in trypan blue. Direct PFC

were enumerated on a lawn of BrMRBC using the method of Cunningham and Szenberg (22). BrMRBC were prepared as described by Bishop and Haughton (16).

Results

Normal Mice Contain a Large Number of Lymphocytes Capable of Recognizing PtC. We have suggested that the CH series of B cell lymphomas, 6 of 27 of which produce IgM that binds PtC (14), represent neoplastic analogs of normal Ly-1$^+$ B cells (23). To test this hypothesis, we looked in normal tissue known to be rich in Ly-1$^+$ B cells (24) with the expectation that a similarly large proportion might be PtC specific. Fig. 1 shows representative fluorescence histograms from staining 2^a4^b tissue with PtC-containing fluorescent liposomes. Liposome-binding cells are present at high frequency in PerC lymphocytes, where Ly-1$^+$ B cells predominate. In spleen, the frequency of liposome-binding cells is shown to be less than one-tenth that of PerC lymphocytes, and in lymph nodes they are barely detectable.

By flow cytometry, we measured the frequency of liposome-binding lymphocytes in PerC from 38 normal, adult 2^a4^b mice. In Fig. 2 we show how we set our forward-vs.-log 90° light scatter gates to separate PerC lymphocytes from macrophages. In comparison with lymph node cells, most PerC lymphocytes are larger and scatter more light in the forward and log 90° dimensions. The light scatter obtained from liposome-binding cells compared with total PerC is also shown. The liposome-binding lymphocytes are homogeneous with respect to light scatter characteristics. They are shown to be among the largest in PerC, and are larger than all lymph node cells. Most PerC lymphocytes from 2^a4^b mice have a high surface IgM/IgD ratio (Mercolino, T. J., unpublished observation). Taken together, these observations suggest that liposome-binding PerC may be totally included in the Ly-1$^+$ B cell subset. This suggestion is consistent with reports that Ly-1$^+$ B cells resemble plasmablasts (25), and have a characteristically high IgM/IgD ratio (26). Fig. 3 presents the measured number of liposome-binding PerC lymphocytes as a function of mouse age and sex. The mean value for binding observed in these experiments was >5% of the lymphocytes. 1–15% liposome-binding PerC lymphocytes were found in each sample. There was no apparent difference between male and female mice, neither was there any systematic change in the frequency of liposome-binding lymphocytes between 60 and 450 d of age. Three mice, two females and a male, were notable exceptions; in these mice, the measured value for liposome-binding PerC lymphocytes was increased by >2 SD from the population mean. The liposome-binding cells in each

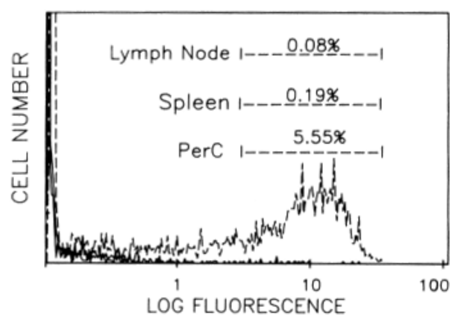

FIGURE 1. Tissue distribution of liposome-binding cells. The dashed line shows the fluorescence intensity distribution, on a logarithmic scale, of lymphocytes in PerC after staining with CF liposomes. Solid lines below show histograms obtained similarly from splenic lymphocytes and lymph node cells. The frequency of cells within the fluorescence intensity range indicated is also given for each tissue. These data come from the tissues of a single mouse, and are representative of all mice tested. These three histograms were collected consecutively at the same fluorescence sensitivity settings.

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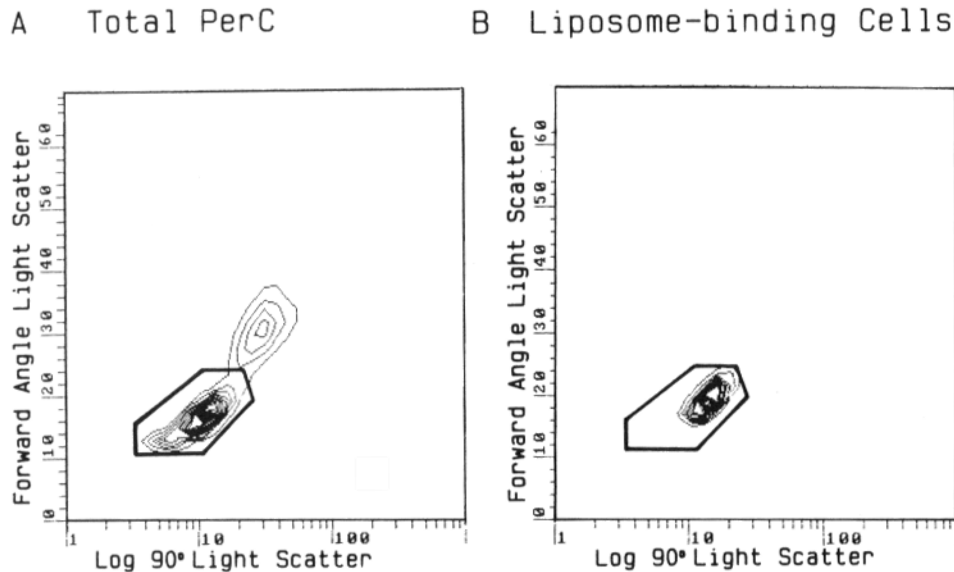

FIGURE 2. Light scatter characteristics of liposome-binding cells. (*A*) Forward angle light scatter-vs.-log 90° light scatter probability density contours for total PerC. The polygon indicates how we set our light scatter gates to exclude PerC macrophages before collecting fluorescence intensity histograms for PerC lymphocytes. Note that within this gate two populations exist with ~90% in the population that scatters more light in the forward and log 90° dimensions. The minor population of smaller cells found in PerC overlaps with respect to light scatter characteristics, and hence size, with the lymph node cells (not shown). To determine the light scatter properties of liposome-binding cells, we analyzed liposome fluorescence-positive cells. (*B*) The liposome-binding cells are totally included in the major, large lymphocyte population in PerC.

of these mice displayed a homogeneous fluorescence profile, an example of which is shown in Fig. 4. Using the peak width at half-height as an estimate of variance in fluorescence intensities measured within each distribution, the sample containing the increased number of liposome-binding cells has a range of 2.1-fold, compared with 3.5-fold for the more typical distribution shown. Three other mice had an obvious homogeneously staining population, yet the total number of liposome-binding cells was within the normal range. Thus, 6 mice, of a total of 22 that were >6 mo old, presented the unusual staining pattern. The homogeneous staining pattern may indicate a narrow range of binding affinity for the liposome probe.

We have studied the ontogeny of cells that bind liposomes, with results shown in Fig. 5. Neither 1-d nor 4-d liver or spleen contained a detectable frequency of cells that bind liposomes ($<10^{-4}$). 8-d-old mice are the youngest mice from which we could obtain PerC. These mice had levels of liposome-binding lymphocytes in PerC of roughly 4×10^{-4}. By 12 d the number had increased 10-fold, and by ~2 wk, half the adult level was reached. Mice between 3 and 5 wk old contained liposome-binding cells at the same frequency as normal adults.

To determine the hapten specificity of PerC that bind liposomes, we performed binding inhibition studies using pure preparations of each of the individual phospholipids used in the probe liposome formulation. As can be seen in Table I, pure PtC effectively blocked the binding of the fluorescent liposomes to PerC lympho-

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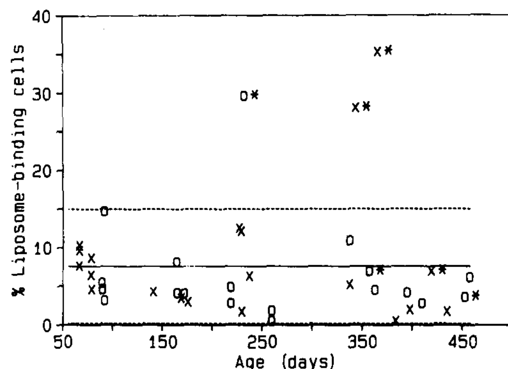

FIGURE 3. Survey of liposome-binding lymphocyte frequency in adult 2^a4^b mice. The percent liposome-binding cells within the lymphocyte light scatter gate for individual mice is plotted as a function of the mouse age in days. The sex of the mouse is indicated for males (O) and for females (X). The solid line shows the mean frequency for all mice, with the dashed lines at ±1 SD from the mean. Three mice are shown to be outliers, and as such have contributed approximately a 2% increase to the calculated mean, and considerably broadened the standard deviation. Notation with an asterisk indicates the presence of what appears to be a clone of liposome-binding cells (see text and Fig. 4).

cytes from 14 of 14 mice. In contrast, phophatidyl glycerol had no effect. We conclude that a single hapten specificity (anti-PtC) accounts for the high frequency of liposome-binding among PerC cells from these normal mice. It is also noteworthy that the mean fluorescence intensity for the anti-PtC cell population is remarkably constant between mice, as is the relative inhibition achieved with pure PtC. We take this to be further evidence indicating similar affinity for PtC in all mice tested.

Virtually All PtC Binders Express IgM and Ly-1. PerC samples were stained with anti-IgM and liposomes. When a Texas red conjugate of affinity-purified goat

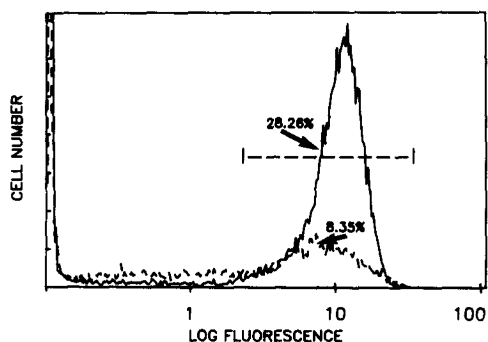

FIGURE 4. Clonal expansion in the liposome-binding cell population. The dashed line shows a frequency distribution of liposome-binding cells from a typical mouse. A representative histogram from an older mouse with an expanded number of liposome-binding lymphocytes is drawn as a solid line. The frequency of cells within the indicated fluorescence intensity range is given for each sample.

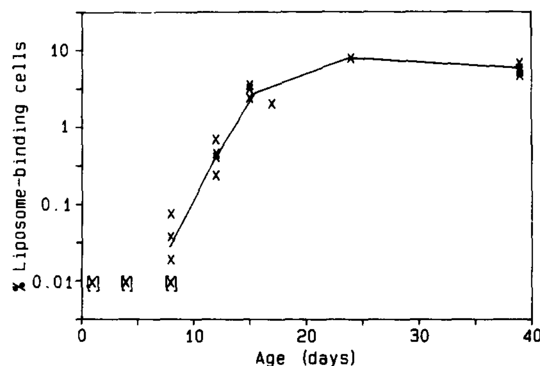

FIGURE 5. Early ontogeny of liposome-binding lymphocytes. Data represent the percent liposome-binding cells in the lymphocyte population (a determined by light scatter) of individual mice plotted as a function of age. Liver and spleen were tested from mice <5 d old; all other samples are PerC. Bracketed data points are below the limit of detection (<0.01%) of our method.

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TABLE I

Liposome-binding PerC Lymphocytes Recognize Phosphatidyl Choline

Exp.	n	Liposome-positive	Relative Fluorescence		
			Control*	PtC block‡	PtG block§
1	9	6.47 ± 1.92‖	1.00 ± 0.15	0.39 ± 0.04	0.98 ± 0.13
2	5	8.71 ± 1.67	1.00 ± 0.20	0.41 ± 0.04	1.02 ± 0.21

* Relative fluorescence values are expressed as a fraction of the mean fluorescence (= 1.00) for the liposome-binding cell population in untreated samples within an experiment.

‡ Samples were preincubated with vesicles of pure PtC before addition of an approximately equimolar amount of PtC/PtG fluorescent liposomes.

§ Samples were similarly preincubated with pure PtG vesicles.

‖ Mean value ± standard error for each measurement.

anti-mouse IgM was used in combination with a fluorescein-containing liposome, the results shown in Fig. 6 *A* were obtained. 70% of PerC lymphocytes were shown to bear surface IgM, and all the liposome-binding cells were included in this population. However, indirect staining with a monoclonal rat anti-mouse μ (clone 331.12), followed by mouse anti-rat κ (MAR 18.5-FITC), completely blocked liposome binding, perhaps due to steric interference by the two added antibodies with the surface IgM molecule. The second-step reagent alone had no effect on liposome binding, nor did any of the other rat antibodies used to phenotype liposome-binding cells (see below). Thus, liposome-binding PerC lymphocytes are B cells, and the blocking result suggests that binding of the probe is by way of surface IgM, as has been demonstrated for the anti-PtC CH lymphomas (14). We sought to determine whether anti-PtC B cells in the peritoneum bear the Ly-1 marker. Using a pool of

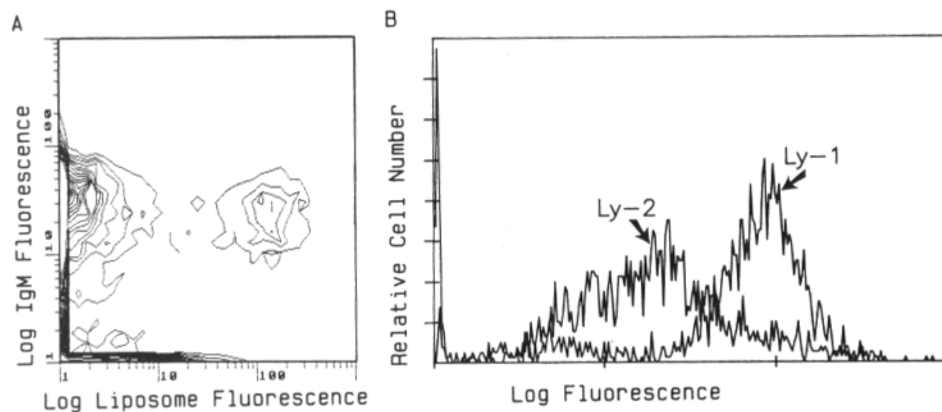

FIGURE 6. Phenotype of liposome-binding lymphocytes. A two-color immunofluorescence analysis of pooled PerC from 5 mice is shown in *A*. Cells were stained simultaneously with CF liposomes and Texas red-conjugated goat anti-mouse IgM. Contour plots drawn at absolute levels of cells with a given staining intensity show that liposome binding cells all bear surface IgM. To generate the histograms shown in *B*, we stained pooled PerC from these same five mice with SRB liposomes, then gated on liposome-binding lymphocytes. Samples stained with liposomes and rat anti-mouse Ly-2/MAR 18.5-FITC resulted in the lower intensity staining profile labeled "Ly-2." Histograms obtained similarly by staining with rat anti-mouse Thy-1.2/MAR 18.5-FITC or with MAR 18.5-FITC alone (not shown) were indistinguishable from that shown for anti-Ly-2. Virtually all cells expressed Ly-1 above this background level, as shown.

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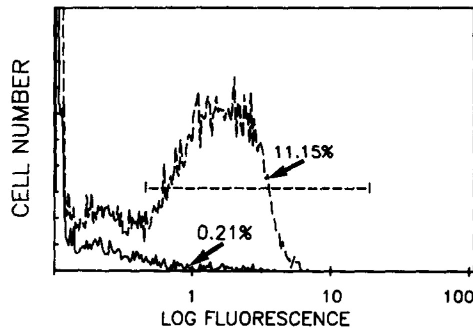

FIGURE 7. Motheaten mice have a low frequency of liposome-binding cells. A fluorescence intensity distribution from a representative individual C3H.*mev/mev* mouse (*solid line*). Virtually all of the B cells from *mev/mev* mice are from the Ly-1+ subset (27). The frequency of liposome-binding cells is seen to be markedly lower than that for a phenotypically normal C3H littermate (*dashed line*).

PerC from five mice, we stained with either rat anti-mouse Ly-1, Ly-2, or Thy-1 and PtC-liposomes. The fluorescence intensity distributions for Ly-1 and Ly-2 expression on liposome binding cells are shown in Fig. 6 *B*. Histograms obtained for Thy-1 staining or for the second-step MAR 18.5-FITC alone (not shown) were indistinguishable from that shown for Ly-2. Ly-1 expression can be seen to be above this background for virtually all PtC-binding cells. The light scatter from liposome-binding cells shown in Fig. 2 *B* indicates a homogeneous, large-cell population.

Me^v Mice Contain Abnormally Low Levels of Anti-PtC Cells. Mice that are homozygous for the viable motheaten gene *me^v* suffer from severe autoimmune disease (18). They have abnormally high numbers of B cells, virtually all of which are of the Ly-1+ B cell subset (27). We wanted to know whether the expanded population of Ly-1+ B cells in young adult C3H *me^v/me^v* mice contained large numbers of liposome-binding cells, or whether the congenital defect interfered with the normal development of these cells. Exemplary data shown in Fig. 7 show that the frequency of liposome binding cells in PerC from 10-wk-old C3H *me^v/me^v* mice was no higher than seen in 8-d-old 2^a4^b mice, whereas PerC from phenotypically normal littermates were within normal range seen for age-matched 2^a4^b mice. We conclude that the expanded population of Ly-1+ B cells in viable motheaten mice does not display the same antigen specificity distribution as the Ly-1+ B cells of normal, young adult mice.

Cells that Secrete Antibody Hemolytic for BrMRBC Are Ly-1+, PtC Liposome-Binding Cells. We sought to determine whether the Ly-1+, PtC liposome-binding cells were the same as, or were the progenitors of, anti-BrMRBC PFC. From a pool of 2^a4^b PerC, we sorted both the lymphocytes that did and those that did not bind CF liposomes. The sorted populations were cultured for 2-4 d in the presence of LPS. In Table II, data from two representative experiments show that essentially all of the precursors to cells that, upon LPS stimulation, secrete antibody hemolytic for BrMRBC are included in the PtC liposome-binding population. The small numbers of PFC generated in the sorted, PtC− population can be accounted for by trace contamination with liposome-binding cells. Three other such experiments gave the same result.

Discussion

In this study, we have demonstrated that fluorochrome-encapsulating liposomes composed of pure, synthetic phospholipids are an extremely sensitive probe capable

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TABLE II
Anti-BrMRBC PFC Are Ly-1+, PtC Liposome-binding Cells

		Population		
Exp.	Culture	I Total*	II PtC−‡	III PtC+§
	d		*PFC/10⁴ cells*	
1	3‖	61¶	3	1,098
	3	38	6	961
2	2	179	2	1,725
	3	198	2	3,353
	4	139	1	3,598

* To obtain population I, total PerC lymphocytes that had been stained with PtC liposomes were sorted only on the basis of the forward-vs.-log 90° light scatter characteristics typical of lymphocytes.

‡ Cells that composed population II were obtained by sorting on the basis of light scatter and the failure to bind PtC liposomes, as determined by log green fluorescence intensity. Reanalysis of sorted cells without restaining showed population II to be consistently >99% negative for liposome binding.

§ Population III were sorted as the PtC liposome-binding lymphocytes. Reanalysis of sorted population III cells showed 70–85% lymphocytes positive for liposome binding.

‖ Sorted cells were placed in culture with 50 μm/ml LPS for the indicated number of days before measuring PFC frequency. The initial culture density of sorted cells for Exp. 1 was 4×10^4/ml, and for Exp. 2, 3×10^5/ml.

¶ Data are means of triplicate determinations from separate cultures.

of identifying very rare cells (0.01%). We have used this technique to detect and analyze lymphocytes that in normal, unimmunized mice, recognize the common membrane phopholipid, PtC. We have found that the peritoneums of normal mice contain many lymphocytes that specifically bind liposomes by recognizing PtC. Using the binding of fluorescent liposomes, these cell could be identified, enumerated, and sorted. After cell sorting and stimulation by LPS, we demonstrated that virtually all peritoneal lymphocytes bearing IgM capable of initiating complement-mediated lysis of BrMRBC also bind liposomes. This implies that lysis of the RBC was a consequence of recognition of PtC exposed by the proteolytic enzyme. Nearly all of these cells bear the cell surface marker, Ly-1.

The findings reported here also have two important technical implications. First is their bearing upon utilization of fluorescent liposomes for cell staining. We described liposomes suitable for use as high signal/noise probes for immunofluorescence staining of cells in the process of defining the anti-PtC specificity of anti-BrMRBC CH lymphomas (14). A similar method has been reported recently (28, 29). The following should be considered in the interpretation of results obtained using antibody-conjugated liposomes intended to specificity stain lymphocytes: (*a*) our present demonstration of a detectable number of PtC-specific cells in both peritoneum and spleen, (*b*) the frequent finding of anti-erythrocyte specificity in human B cell chronic lymphocytic leukemia (B-CLL) (31), and (*c*) that at least one of these Leu-1+ (CD5+) B-CLLs recognizes PtC (Mercolino, T. J., unpublished observation). Second, we believe this to be the initial report of successful sorting of a significant number of B cells with a single antigen-binding specificity from normal, unimmunized mice. Sorting of PtC liposome-binding PerC may prove useful, for

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example, in the study of normal Ly-1$^+$ B cell differentiation, as has been previously described for a transformed clone of Ly-1$^+$ B cells (32).

The experimental findings that PtC liposome-binding cells are present at much higher frequency among PerC than in spleen or lymph node is consistent with a similar distribution of anti-BrMRBC PFC (27); the absolute level of ~7% of PerC is in close agreement with the estimate (33) that roughly 1 in 10 PerC B cells are anti-BrMRBC PFC precursors. The tissue distribution of PtC-binding cells in the adult mouse also reflects the tissue distribution of Ly-1$^+$ B cells (24). Our results support the view that the repertoire of Ly-1$^+$ B cells may be very restricted since, at least in PerC, ~10% of these cells have the anti-PtC specificity. Furthermore, the relative peak position and profile homogeneity of fluorescence intensity for the liposome-binding population is remarkedly similar between mice, as is the degree of inhibition by PtC. We interpret this observation as reflecting similar affinity for hapten in the anti-PtC lymphocyte population. This could be the result of utilization of a small number of Ig variable region gene capable of forming the anti-PtC specificity. The high frequency expression of these gene products in all normal mice tested must mean that, despite their apparent autoreactivity, they perform a beneficial function for the animal. A variety of autoantibody functions have been suggested including the removal of senescent erythrocytes (12) and damaged tissues (34), antibacterial activity (35), and transport of otherwise insoluble material in the serum (36). It is also possible that the products of Ly-1$^+$ B cells are important as a first-line, broadly specific defense that recognizes determinants found on commonly encountered pathogens. Results reported here, taken together with the observation that the serum IgM present in unimmunized mice is produced by Ly-1$^+$ B cells (4), support this view. As such they may represent nonadaptive Ig, the evolutionary predecessors to adaptive antibodies whose specificity is generated, in part, by somatic mutation.

In this study, we also addressed the ontogeny of anti-PtC lymphocytes using the direct enumeration of liposome-binding cells. Our observation of the appearance of PtC-binding cells shows a good correlation with studies of anti-BrMRBC PFC ontogeny (33, 37). The increase in abundance of PtC-specific, Ly-1$^+$ cells during the first 3 wk of life does not result from an increase in the total Ly-1$^+$ B cell population. Indeed, the increase in PtC-specific cells occurs during a period in ontogeny when Ly-1$^+$ B cells are decreasing from the overwhelming majority of B cells in neonatal mice to a minor subpopulation during the next 2–3 wk (26). Thus, the increase in PtC-specific cells reflects a shift in the expressed repertoire of the Ly-1$^+$ B cell population. In marked contrast, we could find no evidence of the normal development of PtC-specific cells in viable motheaten (me^v/me^v) mice. These mice display an extensive overproduction of Ly-1$^+$ B cells for the duration of their shortened life (27). Their severe autoimmune disease may result, in part, from continued, nonspecific proliferation of the Ly-1$^+$ B cell compartment that prevents the repertoire maturation seen in normal mice.

We have also noted a less predictable change in anti-PtC lymphocyte frequency late in life: liposome-binding cells are greatly overrepresented in some mice >6 mo old. Clonal expansion in aging may be a general phenomenon for Ly-1$^+$ B cells (38; 38a). Late in life this expansion seems to reach extremes, with small numbers of clones of cells greatly expanded. Such clonal expression could be a step in neoplastic

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transformation; CD5+ B cells are overrepresented among B cell malignancies in both humans (7, 39) and mice (23, 40).

The expansion of antigen-specific Ly-1+ B cells is presumably controlled. We do not yet understand what this control might be. The predictable repertoire maturation, together with the evidence of restricted gene usage associated with the PtC antigen specificity, is consistent with a clonal selection mechanism for the generation of these cells. At present, it is not possible to exclude either an antigen- or idiotype-driven clonal expansion. An idiotype-specific suppressor effect has been suggested for at least one of the idiotypes expressed by the PtC-specific Ly-1+ B cells (16), and selection of the PtC-specific repertoire by antiidiotype has been demonstrated (41).

One feature of Ly-1+ B cells that does seem to be different from conventional B cells is that they can presumably be stimulated to expand in an enviroment that contains very high levels of antigen. These antigen levels would generally be considered tolerogenic for conventional B cells. We have not been able to demonstrate detectable numbers of conventional B cells expressing the PtC specificity. Since we find no reason to propose that this difference is due to an inability of conventional, Ly-1− B cells to express the appropriate Ig genes to encode this specificity, the large expansion of Ly-1+ B cells with this specificity suggests a qualitatively different response to antigen between Ly-1+ and conventional B cells.

Summary

We have found that, in the peritoneums of normal adult mice, 5–15% of lymphocytes bind a fluorescent liposome probe. In ontogeny, cells with this specificity were shown to appear by 8 d after birth, and increase to the adult frequency by 2–3 wk. Some older mice contain an expanded population of these cells. We have shown that liposome binding occurs by cell surface IgM recognizing the common membrane phospholipid, phosphatidyl choline (PtC). Virtually all of these PtC-specific cells bear the cell surface marker Ly-1. Our results indicate that roughly 1 in 10 peritoneal Ly-1+ B cells has this single specificity. We have found that the precursors to all the cells that form plaques on protease-treated autologous erythrocytes (BrMRBC) are included in the PtC-specific population and can be isolated by FACS. We believe this is the first report of sorting large numbers of B cells with a single antigen specificity from normal, unimmunized animals. This method will allow for in vitro and in vivo studies of differentiative and proliferative properties of Ly-1+ B cells, which may help define their role in development and disease.

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