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Peritoneal Ly-1 B cells: genetic control, autoantibody production, increased lambda light chain expression*

Previous studies demonstrate that Ly-1 B cells and their progenitors are clearly detectable in peritoneum in normal mice. In this publication, we show (a) that peritoneal Ly-1 B cells resemble splenic Ly-1 B cells with respect to surface marker expression and functional activity (autoantibody production); (b) that Ly-1 B frequencies in peritoneum are considerably higher than in spleen; and (c) that genetic mechanisms reduce peritoneal Ly-1 B frequencies to minimal levels in SJL-related mice and to below detectability in CBA/N and other mice with the X-linked immunodeficiency (*Xid*). In addition, we show that that peritoneal (and perhaps splenic) Ly-1 B populations demonstrate an unique bias in immunoglobulin commitment. That is, they are selectively enriched for cells that express IgM heavy chains in association with lambda light chains. Thus, as a whole, evidence presented here defines the peritoneum as a tightly regulated lymphocyte compartment that normally houses a large population of mature Ly-1 B cells with distinctive functional properties.

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1 Introduction

Data from previous multiparameter fluorescence-activated cell sorter (FACS) analysis and sorting studies define a subset of murine B cells that expresses the Ly-1 surface glycoprotein in conjunction with IgM, IgD, Ia and other typical B cell markers. These Ly-1-bearing B cells (Ly-1 B) are physically and functionally distinct from other B cells. They express more IgM and less IgD than most other B cells, they are not normally found in lymph node (LN) or bone marrow (BM), they are rare but demonstrable in spleen and they constitute a major fraction of peritoneal B cells [1, 2]. Furthermore, although Ly-1 B seldom produce antibodies to exogenous antigens such as TNP-Ficoll or TNP-KLH, they nonetheless produce most of the commonly studied IgM autoantibodies in normal and autoimmune mice [3].

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Abbreviations: **FACS:** Fluorescence-activated cell sorter **Ly-1 B:** Ly-1-bearing B cells **Ly-1⁻ B:** Ly-1-negative B cells ***Xid*:** *X* chromosome-linked immunodeficiency **PerC:** Peritoneal cells **BrMRBC:** Bromelain-treated mouse red blood cells **PFC:** Plaque-forming cells **Fl:** Fluorescein **Bi:** Biotin **LPS:** Lipopolysaccharide **LN:** Lymph node **BM:** Bone marrow

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The substantive differences between Ly-1 B and the predominant B cell population(s) in mouse spleen and LN foreshadow recent findings demonstrating that Ly-1 B belong to a separate B cell lineage. Adult BM cells typically used to reconstitute B cells in irradiated recipients selectively fail to reconstitute the Ly-1 B subpopulation (which contains the commonly studied autoantibody-producing cells). Peritoneal cells (PerC) from adults, in contrast, selectively reconstitute Ly-1 B. Liver, spleen and BM cells from young mice reconstitute all B cells (including Ly-1 B) and mixtures of adult BM with any of the sources of Ly-1 B progenitors yield purely additive results. These findings assign Ly-1 B to a distinct developmental lineage originating from progenitors that inhabit the same locations as other B cell progenitors in young animals but move to unique location(s) in adults [4]. In this publication, we focus on the properties of the peritoneal Ly-1 B population, which represents the greatest concentration of Ly-1 B cells in the animal.

The peritoneal cavity has long been known to contain populations of macrophages, monocytes and granulocytes that increase markedly in response to i.p. injection of exudate-inducing agents, such as thioglycollate, proteose peptone or mineral oil [5]. The rapidly changing characteristics of these peritoneal exudate cell (PEC) populations have contributed to the impression that PerC populations in general are in constant flux. Thus, even when harvested from unstimulated animals, PerC populations are commonly thought of as essentially random collections of transient cells that just happen to be present in the peritoneum at a particular time.

This reputation for inconstancy, however, is highly undeserved. As we shall show, the composition of unstimulated PerC populations tends to be very similar in adult mice from most mouse strains. In general, B cells comprise roughly half (30–60%) of the harvested PerC, T cells comprise 10–30% and various other types of cells including macrophages and monocytes make up the remainder. Furthermore, there is a characteristic division among the B cells. Half to two thirds of B cells are typical BM-derived B cells that show surface Ig phenotypes comparable to the predominant B cell populations in LN, spleen and peripheral blood. The remainder are derived from cells in the peritoneum itself and show surface Ig phenotypes characteristic of the Ly-1 B population [1].

We capitalize on this relatively high Ly-1 B frequency in PerC populations to further chart the functional characteristics of Ly-1 B and to explore genetic conditions that significantly influence Ly-1 B representation in PerC populations. Thus we show that PerC Ly-1 B produce autoantibodies; that the frequency of cells expressing the lambda light chain is increased in PerC Ly-1 B; that the NZB genetic background elevates PerC Ly-1 B frequencies; that the SJL genetic background substantially reduces these frequencies; and that the X-linked immunodeficiency (*Xid*) results in the complete disappearance of the PerC Ly-1 B population.

2 Materials and methods

2.1 Mice

Most mice analyzed in this report [BALB/cN, SJL, SJA, (BALB/cN × SJL)F_1, BAB/14, CB17, CSW and C57BL/10] were raised from our breeding colony. CBA/Ca and CBA/HN mice were kind gifts from Dr. H. S. Micklem (Immunology Unit, University of Edinburgh, Scotland) and bred in our animal facility. NZB/BinJ mice were purchased from Jackson Laboratory, Bar Harbor, ME. NZB.*Xid* mice, A.TH and A.TL mice, and NFS mice were generous gifts from, respectively, Drs. A. D. Steinberg, R. H. Schwartz and H. C. Morse III at the National Institutes of Health. CBA nu/nu mice were a kind gift from Dr. I. L. Weissman of Stanford University. Either female or male 3-month-old mice were used in most experiments. Female and male (CBA/N × BALB/c)F_1 mice were obtained from Takeda Pharmaceutical Co., Osaka, Japan.

2.2 Preparation of peritoneal washout cells

PerC were obtained by injecting 8–10 ml of chilled medium (RPMI 1640 without biotin (Bi), riboflavin, phenol red; Irvine Sci., Santa Ana, CA) containing 3% newborn calf serum into the peritoneal cavity of unprimed mice and immediately harvesting peritoneal fluid. Ninety percent of injected volume was usually recovered in this procedure. Samples detectably contaminated with blood were not analyzed. Cells were passed through nylon mesh and washed twice with the same medium.

2.3 Antibodies and reagents

All rat monoclonal antibodies, anti-IgM (331.12) [6], anti-Ly-1 (53-7.3) [7], anti-Lyt-2 (53-6.7) [7], and anti-mouse κ (187.1) were purified from supernatant cultured in serum-free medium (HB101; Hana Media Inc., Berkeley, CA). The rat anti-mouse κ cell line originally established by Dr. Yelton [8] was obtained from American Type Culture Collection (Rockville, MD) and adapted to growth in serum-free medium. Mouse monoclonal antibodies, anti-Igh-5^a (IgD^a, 10-4.2) [9], anti-λ1 (Ls136) [10] were purified from ascites. Fluoresceination and biotination of antibody and Texas Red (TR; Molecular Probes Inc., Junction City, OR) conjugation to avidin (Vector, Berkeley, CA) have been described previously [1].

Anti-Ly-1 and anti-Lyt-2 antibodies were employed as $F(ab')_2$ reagents to avoid labeling Fc-binding cells. They were prepared by pepsin digestion following the method of Rousseaux et al. [11].

Fluoresceinated goat anti-λ antibody was a kind gift from Dr. A. Radbruch (Institute of Genetics, Köln, FRG). This antibody was obtained from a goat immunized with B1-8 (IgM,λ1). The serum was absorbed on a TEPC 183 (IgM,κ) column, and the effluent was then affinity purified on a HOPC 1 (IgG_{2a},λ)-Sepharose column. The purified material was further absorbed with Ac146 Sepharose (IgG_1,κ) yielding a reagent specific for λ-bearing immunoglobulins.

2.4 Staining procedure and data analysis

The staining procedures and data analysis methods used here have mostly been described previously [1]. In essence, cells were stained in microtiter wells and monoclonal antibody reagents labeled with either fluorescein (Fl) or biotin (Bi) (for use with Texas Red-coupled avidin). Analyses were performed with a modified dual-laser FACS II (Becton Dickinson, Sunnyvale, CA) fitted with a second scatter channel to detect scattered light from cells at large angles (obtuse scatter) [12]. This instrument was linked to a VAX 11/780 computer that allows direct acquisition of list mode data and subsequent analyses.

Data from two-color analyses are presented in FACS contour plots computed such that an equal percentage of the cells in the analysis falls between each pair of neighboring contour lines. This "probability contour" plotting method results in plots in which the density of contours in a given area accurately reflects the concentration of cells in that area [13]. Fluorochrome measurements are made with logarithmic amplifiers and are always plotted as the log of the amount of cell-associated fluorescence.

2.5 Scatter gating and analysis for PerC

PerC harvested from unstimulated BALB/c mice show a typical small angle (forward) and obtuse light scatter pattern that effectively distinguishes nonlymphocytic cell populations such as granulocytes and monocytes from the bulk of the lymphocytes present in the suspension. Thus, in analyzing PerC populations, we acquire list mode data for all live cells in the sample (dead cells were gated out as propidium iodide-stained cells) but present contour plots based on data from cells that fall within the appropriate small-angle and obtuse scatter gates for peritoneal lymphocytes (see Fig. 1 and Sect. 3).

2.6 Anti-bromelain-treated mouse red blood cells (BrMRBC) plaque-forming cells (PFC)

C57BL/10 mice were used as the source of MRBC. The bromelain treatment of MRBC and the PFC procedure were described previously [3]. Anti-BrMRBC in PerC was induced by the 2-day incubation of 10^6 PerC in medium RPMI 1640, with 10% fetal calf serum plus 50 μM 2-mercaptoethanol in the presence or absence of lipopolysaccharide (LPS) from *Salmonella typhi* (Difco, Detroit, MI) at 20 μg/ml.

2.7 Identification of phagocytic cells

The phagocytic potential of cells was assessed by measuring the ingestion of 0.75 μm uniform fluorescent latex beads (microspheres; Polysciences Inc., Warrington, PA). Cells were incubated in RPMI 1640 medium containing microspheres with 10% fetal calf serum at 37°C for 30 min. After three washes, the cells (FACS-sorted Ly-1 B and Ly-1^- B cells) from PerC were examined for internally ingested fluorescence beads under the fluorescence microscope.

2.8 Nonspecific esterase staining

Nonspecific esterase staining by α-naphthyl acetate and FAST Blue RR Salt (Sigma, St. Louis, MO) was carried out following a standard method described elsewhere [5].

3 Results and discussion

3.1 Ly-1 B are enriched in PerC populations

PerC populations recovered from normal unstimulated adult mice contain mainly lymphocytes. In general, nonlymphocytic nucleated cells (macrophages, monocytes, polymorphonuclear leukocytes, etc.) account for less than 30% of the total cell yield while lymphocytes account for > 70%. Among lymphocytes, B cell frequencies tend to exceed T cell frequencies by about 2 : 1, depending (as we will show) on the age and the genetic background of the PerC donor.

Although relatively few in number, the nonlymphocytic cells recovered in PerC populations create a problem for FACS analyses. They tend to be autofluorescent and/or to nonspecifically bind fluorochrome-conjugated monoclonal antibodies (data not shown). However, as Fig. 1 shows, these cells can be readily gated out of FACS analyses because the large-angle light scatter signals they generate are substantially higher than signals generated by Ly-1 B and other lymphocytes. Thus, all immunofluorescence studies presented here are based on FACS data gated as shown in Fig. 1 to exclude nonlymphocytic cells.

FACS contour plots showing the correlated expression of IgM and Ly-1 in PerC populations clearly reveal a large population of Ly-1 B (see Fig. 2) The magnitude of this population is somewhat surprising since Ly-1 B are not detectable in normal LN and seldom represent more than 5% of the B cells in spleen [1] or peripheral blood (see Fig. 3). Nevertheless, except in certain mouse strains (see below), Ly-1 B constitute a third to a half of the IgM-bearing (B) cells (roughly 10–20% of total cells) in harvested PerC suspensions.

3.2 PerC Ly-1 B are similar to splenic Ly-1 B

Like its splenic counterpart, the PerC Ly-1 B population expresses low levels of surface IgD and high levels of surface IgM. This conclusion is deducible from the data shown in the IgM/Ly-1 and IgM/IgD contour plots in Fig. 2. In addition, it is directly demonstrated by data from four-color immunofluorescence analyses in which surface Ig expression was measured on cells that bound anti-IgM, anti-IgD and anti-Ly-1 but did not bind the control (anti-Lyt-2) reagent presented

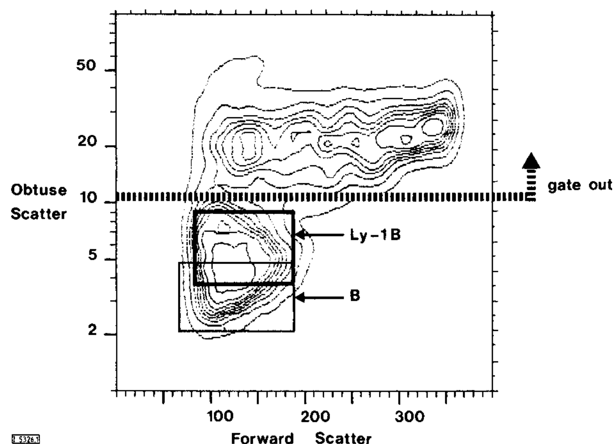

Figure 1. Scatter gates exclude peritoneal macrophages and nonlymphocytic cells. This figure shows the forward and large angle (obtuse) scattered light distribution for a representative PerC suspension (from a 4-week-old BALB/c mouse). In the figures that follow, the cells that give high obtuse scatter signals were excluded (gated out as shown here) to reveal peritoneal lymphocyte populations for analysis. The boxes that enclose the B cell populations were defined by determining the scatter distributions of cells that stain appropriately with anti-Ly-1, anti-IgM and anti-IgD. As indicated, Ly-1 B routinely give somewhat higher obtuse scatter signals than other B cells.

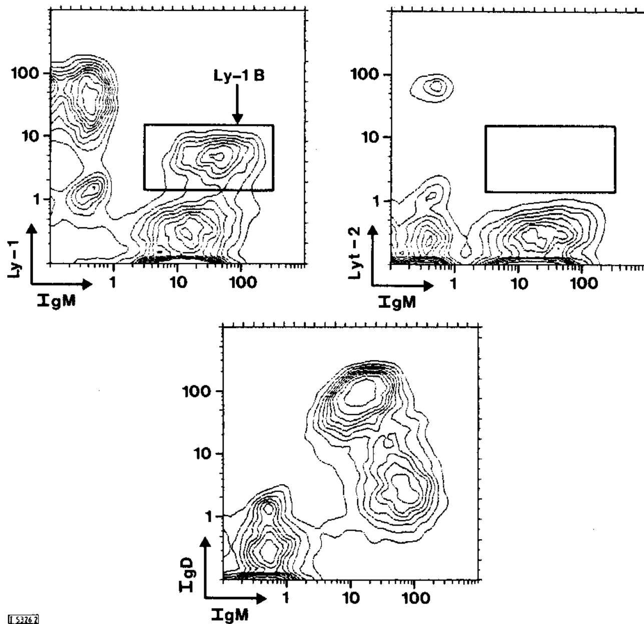

Figure 2. Ly-1 B are clearly demonstrable in PerC populations. PerC from 3-month-old BALB/c mice were simultaneously stained to reveal the cell surface determinants indicated for each set of axes as follows: upper left, fluorescein (Fl)-anti-IgM and biotin (Bi)-anti-Ly-1; upper right, Fl-anti-IgM and Bi-anti-Lyt-2; lower center, Fl-anti-IgM and Bi-anti-IgD. The binding of Bi-conjugated antibody to cells was revealed by staining with Texas Red (TR)-avidin. The box shows the integration gates used to determine Ly-1 B frequencies. The frequency of cells within these gates in a population stained with a comparably prepared control reagent (anti-Lyt-2) was subtracted as background.

together with the other three reagents (data not shown). Thus, Ly-1 B are mainly found in the dull IgD, bright IgM B cell population which, because of its higher frequency in PerC, is clearly visible in the IgM/IgD contour plot shown at the bottom of Fig. 2.

PerC Ly-1 B are similar in size and morphology to the splenic Ly-1 B described previously [1], *i.e.*, they are somewhat larger than most Ly-1⁻ B cells and are lymphoblastoid in morphology. They express typical B cell antigens such as Ia, ThB, B220, IgM and IgD in conjunction with Ly-1 but do not express detectable levels of any of the other commonly studied T cell antigens (*e.g.*, Lyt-2, Thy-1 or L3T4). Furthermore, they differ from peritoneal macrophages in several ways: (a) they are not phagocytic; (b) they tend to adhere to glass or plastic slightly more than other B cells when incubated for 24 h at 37 °C in medium containing 10% serum, but do not adhere as strongly as macrophages; and (c) their nonspecific esterase staining (strongly positive in monocytes) is negative or only slightly positive.

3.3 Relative frequencies of PerC Ly-1 B are highest in young animals

Significant numbers of Ly-1 B are often present in the peritoneum as early as 7 to 10 days after birth, at a time when other B cells are only marginally detectable. Three weeks after birth, Ly-1 B frequencies have increased to approximately 25% of the stable adult level while other B cells have only reached about 10% of adult levels (Fig. 4). This early enrichment for Ly-1 B in PerC B cell populations is similar to the

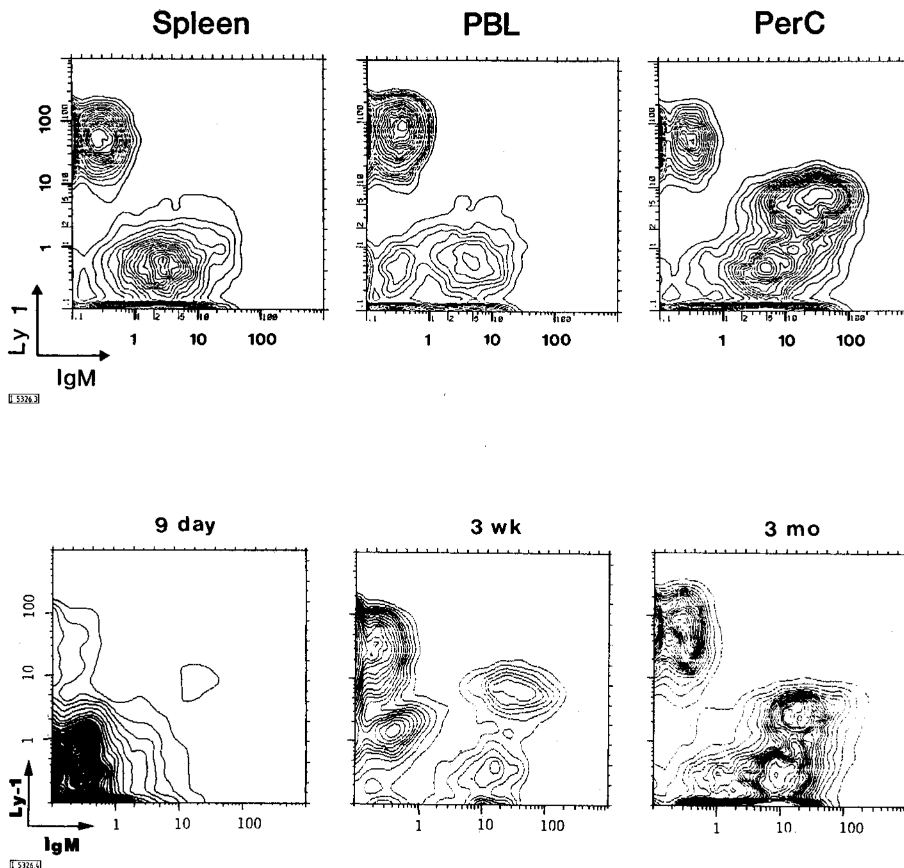

Figure 3. PerC has more Ly-1 B than spleen or peripheral blood. A 3-month-old BALB/c mouse was killed for the preparation of spleen cells, peripheral blood lymphocytes (PBL) and PerC. Cells were stained with Fl-anti-IgM and Bi-anti-Ly-1. The frequency of Ly-1 B was 2% in spleen, 2% in PBL and 17% in PerC.

Figure 4. The predominance of Ly-1 B cells in the peritoneum begins early in life. Peritoneal cells from mice at the indicated ages were stained with Fl-anti-IgM and Bi-anti-Ly-1. Data shown are from PerC from a pool of eight 9-day-old mice, a pool of three 3-week-old mice and a pool of two 3-month-old mice. The recovered numbers of PerC per animal in each of the groups were 4×10^5, 1×10^6 and 5×10^6, respectively. The respective frequencies of Ly-1⁻ B and of Ly-1 B were as follows: 1% and 2% at 9 days of age (Ly-1⁻ B cells were not well resolved); 5% and 4% at 3 weeks of age; and 19% and 37% at 3 months of age.

early enrichment for Ly-1 B shown previously in splenic B cell populations in neonatal animals [1].

PerC Ly-1 B frequencies continue to rise as young animals mature into adulthood; however, the frequencies of the other B cell populations rise somewhat more rapidly. Therefore, by the time stable adult PerC B cell levels are established (at about 3 months of age), Ly-1 B represent between one third and one half of the B cells present. Older animals (over 8 months of age) sometimes show increases or decreases in Ly-1 B frequencies but these variations tend to occur only in certain strains, and the direction of the change tends to be characteristic of the strain (*e.g.*, PerC Ly-1 B frequencies often increase in older NZB mice).

3.4 Genetic control of Ly-1 B frequencies

Although Ly-1 B frequencies normally tend to be similar in PerC populations from adult mice, PerC populations from animals with certain genetically controlled immune defects show characteristic frequency shifts. That is, NZB-related mice routinely show increased Ly-1 B frequencies in PerC; SJL-related mice, in contrast, show decreased but clearly detectable PerC Ly-1 B frequencies; DBA/2Ha mice, recently shown to be immunodefective [14], completely lack Ly-1 B in PerC; and, similarly, CBA/N and other mice with *Xid* show no detectable PerC Ly-1 B (Table 1).

The defect in SJL-related mice is most pronounced in inbred (homozygous) animals, *i.e.* in SJL and its allotype congenic strain, SJA, which carries the Igh^a rather than the Igh^b chromosomal region. On average, PerC Ly-1 B frequencies in these animals are nearly an order of magnitude lower than the PerC Ly-1 B frequencies in BALB/c and other normal mice. Ly-1 B levels in F_1 hybrids between SJL and BALB/c, in contrast, lie midway between the two parental frequencies (Table 2). Furthermore, levels in males and females from these strains and crosses are indistinguishable (data not shown). Thus, PerC Ly-1 B frequency differences in SJL and BALB/c mice are controlled by autosomal gene(s) that are co-dominantly expressed in heterozygotes and operate independently of the Ig heavy chain allotypes expressed in the animal.

The PerC Ly-1 B deficit in *Xid* mice, in contrast, is controlled directly or indirectly by sex-linked gene(s) that are identical or closely linked to the gene(s) responsible for the overall immunodeficiency in these mice. As Table 2 shows, there are no detectable peritoneal Ly-1 B in CBA/N (*Xid* homozygous) animals or in F_1 male progeny from CBA/N females outcrossed to BALB/c males; however, female litter mates from this same (CBA/N × BALB/c) cross have normal PerC Ly-1 B levels. Therefore, alleles of at least two unlinked loci (one autosomal and one sex-linked) contribute to the control of Ly-1 B frequencies in the peritoneum.

Curiously, splenic Ly-1 B frequencies appear normal in *Xid*, SJL-related and DBA/2Ha mice (data not shown) despite

Table 1. Strain differences in Ly-1 B frequencies in peritoneum

Ly-1 B frequencies Level	Range[a)] (%)	Strains tested[b)]
High	25–70	NZB
Moderately high	10–35	BALB/c, BAB/14, C.B17, BALB.Hi[c)]
Intermediate	5–20	CBA, CBA.Igh^b, CBA nu/nu, NFS, CSW, C57BL/10, A.TH, A.TL, 129/SV[d)]
Low	<1–6	SJL, SJA
Undetectable	<1	CBA/N, NZB.*Xid*, DBA/2Ha[e)]

a) Percent in harvested peritoneal cells (see Table 2 for details).
b) Mice were tested at 2–4 months of age.
c) BALB/c mice congenic for high ThB expression. ThB allele from SJL mice [15] (established in our animal facility).
d) From Dr. Van Snick (UCL-ICP, Bruxelles, Belgium).
e) From Dr. T. Hamaoka (Osaka University, Japan).

Table 2. Genetic control of peritoneal Ly-1 B frequencies

Strain[b)]	No. of mice tested	Cells recovered $\times 10^{-6}$	Peritoneal cell frequencies[a)] All IgM^+ (%) range	Ly-1 B (%) range	T cells[c)] (%) range
BALB/c	22	5 (3–7)	60 (48–73)	18 (10–27)	16 (11–24)
(BALB/C × SJL)F_1	14	6 (3–8)	53 (35–67)	8 (3–13)	28 (17–40)
SJL	10	5 (2–9)	48 (29–64)	2 (<1–6)	29 (18–41)
CBA	6	5 (5–6)	37 (23–53)	12 (7–18)	14 (8–20)
(CBA/N × BALB/c)F_1 ♀	4	4 (2–5)	36 (30–40)	13 (10–16)	12 (7–14)
(CBA/N × BALB/c)F_1 ♂	4	1 (1–2)	19 (16–23)	<1[d)]	12 (8–16)
CBA/N	6	2 (1–3)	11 (6–22)	<1[d)]	29 (25–43)

a) Mean and range of frequency (in parentheses) obtained from tested mice are presented in this table.
b) Three–4-month-old mice were tested.
c) T cells were defined as $Ly\text{-}1^+$ IgM^- cells.
d) No more than background staining (by anti-Lyt-2; 0.2–1%).

clear-cut peritoneal Ly-1 B deficits demonstrated at the same time in the same animals. This suggests either that PerC and splenic Ly-1 B frequencies are controlled independently or that FACS measurements of splenic Ly-1 B frequencies are too imprecise to detect reduced Ly-1 B levels. Functional studies presented next bring more evidence to bear on this point.

3.5 Autoantibody production (BrMRBC PFC) measures Ly-1 B representation

PFC that produce IgM autoantibodies to determinant(s) revealed on BrMRBC are routinely detectable at low frequencies in unstimulated mice and at substantially higher frequencies in LPS-stimulated animals [16]. These autoimmune PFC have long been known to have many of the properties we have now demonstrated for FACS-detected Ly-1 B cells. They tend to be absent from LN, present in spleen and enriched in the peritoneum in normal animals [17]. Furthermore, they tend to be overabundant in NZB-related mice and deficient in *Xid* animals [18]. Thus, not surprisingly, spontaneous and LPS-induced anti-BrMRBC PFC are almost all contained within FACS-sorted Ly-1 B populations [3].

BrMRBC PFC studies further define the genetically controlled Ly-1 B deficiencies demonstrated above by FACS analysis of PerC (see Sect. 3.4). The two methods yield essentially equivalent data for PerC populations, *i.e.*, CBA/N have no FACS-detectable Ly-1 B and show no detectable anti-BrMRBC PFC, SJL-related mice show lower PFC levels and lower Ly-1 B frequencies, and normal mouse strains show high PFC and high PerC Ly-1 B levels (compare Tables 1 and 3). However, while FACS analyses fail to reveal genetically controlled Ly-1 B deficiencies in spleen, functional (anti-BrMRMC PFC) analyses reveal these deficiencies quite clearly. Thus, as data in Table 3 show, CBA/N mice lack splenic anti-BrMRBC PFC and SJL mice have significantly fewer splenic PFC, even after *in vivo* stimulation with LPS.

There is no obvious explanation for the presence of apparently normal numbers of FACS-detectable Ly-1 B cells in spleen cell suspensions from genetically defective animals. FACS estimates of splenic Ly-1 B frequencies are admittedly approximate, since Ly-1 B seldom exceed 2% of spleen cells in suspension (except in NZB-related animals); however, the inaccuracies of FACS frequency measurements in this range are unlikely to be great enough to account for the numbers of Ly-1 B detected since splenic Ly-1 B deficiencies are recognizable under other circumstances, *e.g.*, in BM repopulated animals [4]. Alternatively, the FACS frequency measurements may reflect the presence of nonfunctional or differently functional Ly-1 B in spleen that are not controlled by the genetic mechanisms discussed here.

3.6 PerC Ly-1 B are enriched for cells producing λ light chains

Although cells producing λ light chains are usually quite rare in murine B populations (up to 5%), roughly 20% of Ly-1 B in the peritoneum express λ rather than κ light chains. Other (Ly-1⁻) PerC B cell populations do not show this increase in λ expression and have essentially the same frequency of λ-bearing cells as the predominant B cell populations in spleen. The enrichment for cells expressing λ chains in the PerC Ly-1 B population can be revealed either as an excess of cells specifically stained with both anti-λ and anti-Ly-1 reagents (Table 4 and Fig. 5) or as an equivalent deficit of anti-κ stained cells in the PerC Ly-1 B population (data not shown). Simultaneous staining with the anti-κ and anti-λ reagents does not reveal significant numbers of cells expressing both light chains (Fig. 5). Splenic Ly-1 B could also be enriched for λ-expressing cells; however, the extremely low frequency of Ly-1 B in spleen precludes accurate analysis of this population for λ expression. Thus, the mechanism(s) that drive or support λ expression in murine B cells apparently operate more efficiently or more actively in PerC Ly-1 B and perhaps in all Ly-1 B.

The distribution of λ subtypes in PerC Ly-1 B tends to vary from animal to animal. Cells expressing λ1 sometimes pre-

Table 3. PerC and spleen anti-BrMRBC PFC frequencies are reduced in SJL and CBA/N mice

Mice	Mitogen	Anti-BrMRBC PFC PerC[a)]	Spleen[b)]
BALB/c	–	222 ± 55	198 (91–314)
SJL	–	48 ± 24	<9 (<1– 35)
CBA/N	–	<1	<1
BALB/c	LPS	2256 ± 358	762 (419–1187)
SJL	LPS	245 ± 131	28 (10– 43)
CBA/N	LPS	<5	<8 (<1– 24)

a) One million peritoneal washout cells (PerC) were cultured in the presence or absence of LPS (20 µg/ml). Cells were harvested two days after culture and assayed for PFC. Data are presented as an average of PerC PFC from three individual mice from each strain.
b) Spleen cells (10^7) from unprimed or LPS injected (10 µg i.p. 2 days previously) mice were tested for anti-BrMRBC PFC frequency. Eight to 10 mice of each group were examined. The mean and individual range in each group are shown.

Table 4. Lambda-bearing cells are enriched in PerC Ly-1 B

BALB/c mice[b)]	Ly-1⁺ B[a)] in PerC λ⁺ (%)	μ⁺ (%)	λ⁺/μ⁺ × 100	Ly-1⁻ B[a)] in PerC λ⁺ (%)	μ⁺ (%)	λ⁺/μ⁺ × 100
# 1	2	11	18	2	36	6
# 2	2	11	18	2	48	4
# 3	3	31	10	1	33	3
# 4	3	14	21	2	29	7
# 5	3	15	20	3	47	6
# 6	4	33	12	1	27	4
# 7	5	38	13	2	27	7
# 8	5	28	18	2	35	6
# 9	5	21	24	2	40	5
#10	6	22	27	1	34	3
Mean	4	22	18	2	36	5

a) Ly-1⁺ B are defined as IgM⁺, Ly-1⁺ cells; Ly-1⁻ B cells are defined as IgM⁺, Ly-1⁻ cells. The frequency of total Ly-1 B and λ-bearing Ly-1 B was obtained by subtraction of the frequency of "background" staining defined by binding with Bi-anti-Lyt-2 (0.2–1%).
b) Peritoneal cells from 3–4-month-old BALB/c mice were stained with Bi-anti-Ly-1 (plus TR-avidin) together with either Fl-anti-IgM or F1 anti-λ.

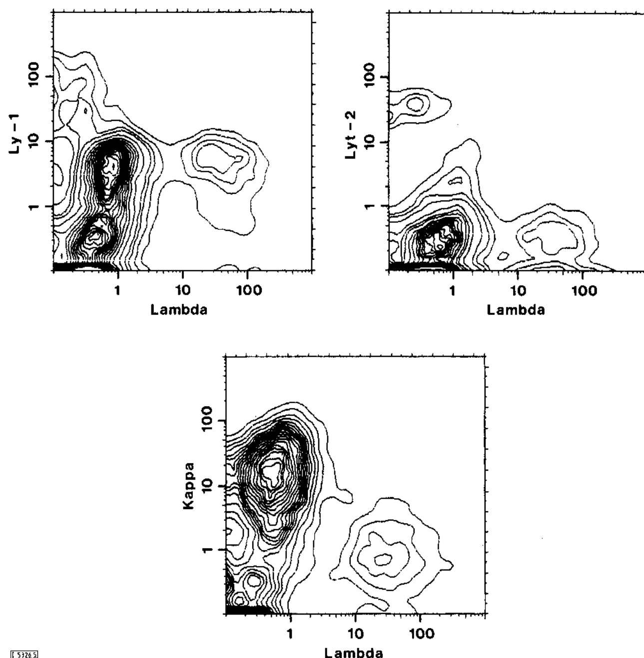

Figure 5. Ly-1 B are enriched for cells bearing λ Ig light chains. PerC from 3-month-old BALB/c mice were stained with Fl-goat anti-λ and Bi-anti-Ly-1 (upper left); Fl-goat anti-λ and Bi-anti-Lyt-2 (upper right); and Fl-goat anti-λ and Bi-anti-κ (lower center). Frequencies of Ly-1$^+$,λ$^+$ cells and Ly-1$^-$, λ$^+$ cells were 4% and 1%, respectively. The contour plot at the bottom shows that PerC B cells carry either κ or λ light chains, not both.

dominate markedly (as they nearly always do in spleen); however, in general, there tends to be a more equal representation among the three λ subtypes such that λ1-expressing cells not infrequently constitute a minority of the total λ-expressing population (data not shown). Thus, on the whole, the PerC Ly-1 B population appears to be considerably more varied in its λ chain constant region expression than B cell populations in other locations or than other B cell populations in PerC.

3.7 The origin of PerC lymphocytes

We have not been able to identify any specific peritoneal structure that houses the lymphocytes washed from the peritoneum. None of the lymphoid organs in the peritoneum (Peyer's patches, mesenteric nodes, omentum, spleen) show any evidence of being disrupted by the washing procedure. Furthermore, with the exception of the spleen, none of these organs have detectable numbers of Ly-1 B (data not shown). Therefore, PerC populations are either derived from an as yet unknown lymphoid organ or are composed of essentially free-living cells whose representation in the peritoneum is regulated by mechanisms perhaps similar to those that regulate the frequencies of the various lymphocytes in peripheral blood.

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