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CD43 (S7) Expression Identifies Peripheral B Cell Subsets[1]

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CD43 (leukosialin) expression has previously been demonstrated on the surface of developing B cells in mouse bone marrow and on plasma cells induced in vitro, but not on peripheral B cells in spleen. Here we show that CD43, as recognized by mAb S7, is indeed expressed on a small population of splenic B cells. Flow cytometric phenotyping of normal mice and radiation chimeras reveals that CD43/S7 is expressed on virtually all (>90 to 95%) splenic B-1 cells and the majority of peritoneal B-1 cells, but not on conventional B cells. The expression of CD43/S7, in conjunction with other cell surface markers, clearly distinguishes B-1 cells from follicular, marginal zone, and immature B cells in the unstimulated adult spleen and permits further phenotyping of these subsets. The phenotype of splenic and peritoneal B-1 cells in normal BALB/c and BAB/25 mice is essentially identical with the exception that all peritoneal B-1 cells express CD11b (Mac-1) and some lack CD43/S7 and heat stable Ag (as detected by the mAb 53-10) expression. Although splenic B-1, marginal zone, and immature B cells share many phenotypic characteristics, these studies show that, in addition to CD43, they differ with respect to the expression levels of a variety of Ags including heat stable Ag, B220, and the B cell activation Ag B7. *The Journal of Immunology*, 1994, 153: 5503.

CD43 or leukosialin, initially identified as a major glycoprotein on mouse, rat, and human T cells (1–3), is also present on granulocytes as well as on a subpopulation of B lineage cells (1, 4). Although the function of CD43 is poorly understood, this highly sialated molecule might be important in the stimulation and proliferation of T cells, possibly via the same signal transduction pathways that are involved in T cell activation through the CD3 complex (5, 6).

In mice, the rat mAb S7 (1) recognizes a CD43 epitope that is present on early B cell precursors, but becomes lost as these cells differentiate to pre-B and B cell stages (4). CD43/S7 was not observed on splenic B cells; however, in vitro stimulation of splenic B cells with LPS induces high levels of CD43/S7 expression during the terminal phases of B cell differentiation, e.g., on plasma cells (1). Here we use multi-parameter FACS analysis to examine CD43/S7 expression on peripheral B cells in spleen and peritoneum.

B cells can be divided into two phenotypically and functionally distinct populations (7, 8) referred to as conventional (or B-2) and B-1 (Ly-1 B) cells (9). It is generally accepted that these two populations represent at least two discrete B cell lineages (7, 10–13). In the unstimulated adult spleen, conventional B cells predominantly consist of follicular B cells plus two smaller subsets, namely, marginal zone (MZ)[4] and immature B (IB) cells. In adult spleen, B-1 cells represent only a few percent of total B cells (7); however, the absolute number of B-1 cells ($\approx 5 \times 10^6$) is equivalent to that found in the PerC where they constitute 40 to 80% of the B cells (11, 14). Although B-1 cells represent a small fraction of total B cells, studies indicate that B-1 cells produce at least 50% of natural serum IgM in normal mice (15, 16). The location of the cells that are actively producing this Ab is unclear. Studies have shown that PerC B-1 cells have a low turnover rate (17) and low spontaneous Ab production as measured in plaque-forming cell assays (S.M.W. and A.M.S., unpublished observation), but after adoptive transfer, B-1 cells

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[4] Abbreviations used in this paper: MZ, marginal zone; APC, allophycocyanin; B-CLL, B cell chronic lymphocytic leukemia; BM, bone marrow; Fl, fluorescein; HsAg, heat stable Ag; IB, immature B cell; PE, phycoerythrin; PerC, peritoneal cavity; PI, propidium iodide; TR, Texas Red.

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yield a large fraction of the "plasma cell-like" cytoplasmic IgM$^+$ cells found in the spleen (18). These facts suggest that the splenic B-1 cells and their descendent plasma cells are in fact the more functionally active subset of this population.

The B-1 population was originally defined by the expression of CD5 (19, 20); however, another population of B cells is now recognized which, except for the expression of CD5, shares most of the phenotypic and functional properties of B-1 cells, most notably self-replenishment and feedback regulation (7, 11, 21). These two subpopulations are now designated as B-1a (CD5$^+$) and B-1b (CD5$^-$) (9, 11, 22). All B-1 cells express high levels of IgM, low IgD, low B220 (as detected by the MAb RA3-6B2), and no detectable CD23 (Fc_eR) (23). In the PerC, B-1 cells are also easily distinguished from conventional B cells by their expression of the Ag Mac-1 (CD11b). It is more difficult to distinguish B cell subsets in the spleen. B-1b cells share many phenotypic characteristics with MZ B cells (23) and IB cells (24, 25), which express similar levels of IgM, IgD, and CD23. Although B-1a cells can be distinguished from B-1b, IB and MZ B cells by the expression of CD5, quantification of total B-1 and in particular B-1b cells is further complicated because, unlike those B cells found in the peritoneum, splenic B-1 cells do not express detectable surface CD11b (11, 22). This lack of cell surface Ags that distinguish among splenic IB, MZ B cells, and both B-1a and B-1b cells (in addition to their low frequency) has made it extremely difficult to definitively study splenic B-1 cells.

We show in this paper that CD43/S7 is expressed on most PerC B-1 cells and essentially all splenic B-1 cells; however, it is absent on all splenic conventional B cells, including MZ and IB cells. The expression of this cell surface Ag has enabled us to distinguish between MZ B cells and B-1 cells in unstimulated spleen, and more importantly, to distinguish and study pure populations of splenic B-1 cells. Using CD43 to distinguish B-1 cells, we have studied the frequency of splenic B-1 cells in mice of varying ages as well as of different strains. In addition, a phenotypic analysis has allowed the first detailed comparison of the expression levels of many cell surface Ags on splenic and PerC B-1 cells.

Materials and Methods

Mice

CBA/Ca, C57BL/6, BALB/c (Igh-C^a) and allotype congenic BAB/25 (Igh-C^b) mice were bred and maintained in the animal facilities at either Columbia or Stanford Universities.

mAbs

Mouse monoclonal anti-Igh-6a (IgM^a, DS-1), anti-Igh-6b (IgM^b, AF6-78.25), anti-Igh-5b (IgD^b, AF6-122.2), and anti-Igh-5a (IgD^a, AMS 9.1) have been described (26). FITC, PE, and/or biotinylated conjugates of monoclonal anti-HsAg (M1/69), anti-LECAM-1 (MEL-14), anti-I-A^d (AMS 32.1), anti-I-E^k (14-4-4s), anti-H-2K^d (SF1-1.1), anti-CD45 (30F11.1) (27, 28), anti-CD23 (B3B4), anti-Ly-6C (AL-21), anti-LFA-1a (2D7), anti-ICAM-1 (3E2), anti-LPAM-1 (R1-2), anti-Pgp-1 (IM7), and anti-CD3 (2C11) were obtained from PharMingen (San Diego, CA). FITC-conjugated goat anti-human IgG was obtained from CalTag Laboratories (South San Francisco, CA). FITC-conjugated anti-J11d.1 was kindly provided by Dr. Thomas Waldschmidt (University of Iowa, Iowa City, IA). Mouse CTLA-4-human Ig fusion protein was kindly provided by Dr. Lewis Lanier at DNAX Research Institute of Molecular and Cellular Biology, Inc. (Palo Alto, CA). Rat mAbs, anti-CD43 (S7) (1), anti-CD23 (B3B4), anti-IgM (331.12) (29), anti-CD5 (53.7.8) (28), anti-B220 (RA3-6B2), anti-B220 (RA3-2C2), anti-Mac-1 (M1/70) (30), anti-HsAg (BLA-1/53-10) (28), anti-IgD (11-26), anti-CD4 (GK1.5), anti-CD8a (53.7), and anti-Gr-1 (8C5) were purified from supernatants obtained by culture in serum-free medium (HB101; Irvine Scientific, Irvine, CA) and purified by saturated ammonium sulfate precipitation and DEAE ion exchange chromatography. The conjugations of Abs FITC or biotin, and avidin (Vector Laboratories, Inc., Burlingame, CA) to Texas Red (TR; Molecular Probes, Inc., Junction City, OR), have been described previously. The preparations of allophycocyanin (APC) and phycoerythrin (PE) and coupling of APC and PE to mAbs have also been described (11, 31).

Irradiation and transfer

Four- to six-month-old BAB/25 mice were x-ray irradiated with 650 rads 1 day before cell transfer and placed on acidic water (pH 3) containing neomycin sulfate (1 mg/ml, Pharma-Tek, Huntington, NY). Bone marrow (BM) recipients were injected i.v. with 2×10^6 BALB/c cells (femur and tibia). BALB/c peritoneal cells (2 or 3×10^6) were transferred with 2×10^6 syngeneic bone marrow cells as a hematopoetic source. For the transfer of FACS-sorted populations, cell amounts correspond to the equivalent population when unseparated cells were transferred. B-1a/BM chimeras received 9×10^5 sorted B-1a cells (reanalysis of the sort: 98% live B-1 cells; 97% of these were B-1a). B-1b/BM chimeras received 3×10^5 sorted B-1b cells (reanalysis of the sort: 98% live B-1 cells; 86% of these were B-1b). Chimeric mice were analyzed 2 to 5 mo after transfer.

In vitro LPS cultures

Single cell suspensions of BALB/c spleen were prepared as previously described (11). Cells were cultured at 1×10^6 cells/ml in a humidified 37°C, 5% CO_2 incubator for 24 h in RPMI-1640 (Life Technologies, Grand Island, NY), supplemented with 10% FCS (JRH Biosciences, Lenexa, KS), 5×10^{-5} M 2-ME, 2 mM L-glutamine, penicillin G/streptomycin sulfate (Life Technologies) with 20 μg/ml LPS (*Escherichia coli* 0111:B4; Difco, Detroit, MI). Cells were harvested and stained for FACS analysis as described below.

Cell staining and FACS analysis

Three and four-color staining, analysis, and cell sorting previously have been described in detail (11, 32). All experiments were done using 1 μg/ml propidium iodide (PI) to identify dead cells. PI-positive dead cells were excluded in either the PE or TR channels, or where otherwise noted, in a the third fluorescence channel (FL-3-1) from the first (488 nm) laser (32). Cells were analyzed with either a dual laser (argon and argon/dye) FACStar^{Plus} (Becton Dickinson, San Jose, CA) or on Flasher, an extensively modified dual laser FACS II (Becton Dickinson). Data were analyzed using FACS/DESK software (Stanford University, Palo Alto, CA) (32, 33). All the plots presented here have 5% probability contours. Data were collected on 100,000 cells unless noted otherwise.

Results

Majority of peritoneal B-1 cells express CD43/S7

Peritoneal B-1 cells can be distinguished from conventional B cells by surface expression of IgM and IgD (7, 14) as shown in Figure 1. The IgM^{dull}, IgD^{bright} population contains conventional B cells, whereas the B-1 cells are found within the IgM^{bright}, IgD^{dull} population. The center two panels confirm that B-1 cells express minimal or no CD23. The B-1 population contains both B-1a (CD5$^+$)

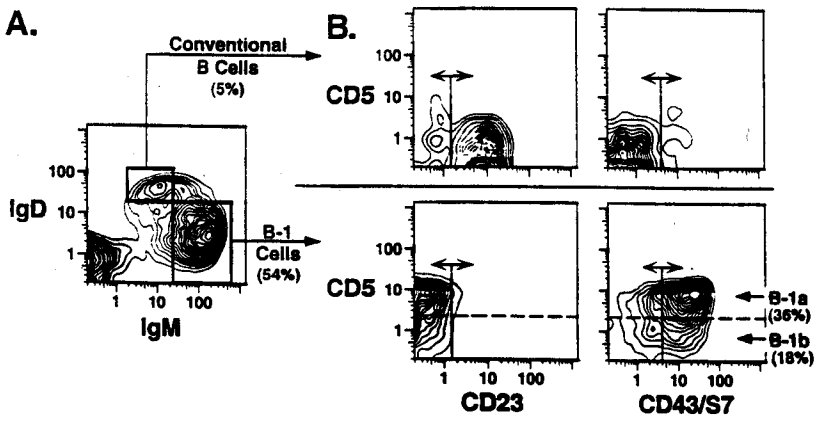

FIGURE 1. Peritoneal B-1 cells express CD43. Peritoneal cells from a 2-mo-old BAB/25 mouse were stained with IgM (Fl), IgD (TR), CD5 (APC) and CD23 (PE) or S7 (PE) mAbs. (*A*) The IgM vs IgD profile of lymphocytes (gated by FSC vs SSC) is shown. The boxes denote the gates for the B-1 and conventional B cells. (*B*) The CD23 vs CD5 and CD43/S7 vs CD5 phenotypes are shown for the gated populations. The gates for CD23 and CD43 used to calculate the values in Table I are shown. The placement of the gates for positive and negative cells were determined by background staining of each mAb on negative populations. Analysis based upon 30,000 cells collected.

Table I. *CD43/S7 and CD5 expression on peritoneal B-1 cells from mice of different ages and strains*[a]

			Percent of B-1 Cells					
			Total		CD43/S7$^-$		CD43/S7$^+$	
Strain (age)	*n*	B-1 Cells	CD5$^-$	CD5$^+$	CD5$^-$	CD5$^+$	CD5$^-$	CD5$^+$
BAB/25 (1 mo)	6	54 ± 3.5	16 ± 2.7	84 ± 2.7	2.0 ± 0.7	1.6 ± 0.5	14 ± 2.1	82 ± 2.9
BAB/25 (3 mo)	4	45 ± 4.8	27 ± 5.5	74 ± 5.0	2.1 ± 0.5	1.2 ± 0.2	24 ± 5.0	76 ± 4.9
BAB/25 (6 mo)	5	61 ± 11.8	49 ± 8.2	49 ± 5.3	11 ± 6.3	4.0 ± 2.7	39 ± 3.8	44 ± 7.6
BALB/c[b] (1 mo)	4	33 ± 9.1	16 ± 4.1	84 ± 4.1	3.9 ± 1.2	13 ± 3.3	13 ± 2.4	71 ± 7.2
BALB/c[b] (3 mo)	4	57 ± 4.9	28 ± 8.4	72 ± 8.4	7.4 ± 5.6	17 ± 2.4	21 ± 3.9	55 ± 11
BALB/c[b] (6 mo)	4	61 ± 11	42 ± 4.1	58 ± 4.1	14 ± 6.8	16 ± 4.1	28 ± 4.8	42 ± 7.5
CBA/Ca (3 mo)	3	46 ± 3.4	39 ± 5.7	61 ± 5.9	9.0 ± 2.1	5.1 ± 0.3	30 ± 3.7	56 ± 5.9
C57BL/6 (3 mo)	5	30 ± 9.1	33 ± 5.9	67 ± 6.1	5.6 ± 4.1	2.3 ± 1.9	27 ± 4.4	65 ± 6.5

[a] Values are given as mean ± standard deviation.
[b] BALB/c mice were from the Stanford colony.

and B-1b (CD5$^-$) cells (22). Conventional B cells, in contrast, are clearly CD23$^+$ (23). Previous studies indicated that CD43/S7 is expressed on early precursors for conventional B cells and again on terminally differentiated B cells, but not on splenic B cells (1, 4). The right-hand panels in Figure 1 clearly show that in the PerC, both B-1a (36% of PerC lymphocytes) and B-1b (18% of PerC lymphocytes) cells are predominantly CD43/S7$^+$, whereas conventional B cells lack detectable CD43/S7 expression. The small number of CD23$^-$ cells in the upper middle panel and CD43/S7$^+$ cells in the upper right-hand panel is most likely contaminating B-1 cells included in the conventional B cell gate, because the frequency can be decreased or increased by tightening or expanding the conventional B cell gate. The gate shown was chosen as the best compromise.

Most peritoneal B-1 cells are CD43/S7$^+$ (Fig. 1). However, there is considerable variation in the percentage of B-1 cells expressing CD43/S7, from less than 60% to greater than 95%. Frequency data presented in Table I indicate that this variation is influenced by age, genetic background, and possibly environment. As the animals age, the proportion of B-1 cells that are CD43/S7$^-$ increases. For example, although less than 4% of the peritoneal B-1 cells in 1-mo-old BAB/25 mice are CD43/S7$^-$, almost 15% of the B-1 cells are CD43/S7$^-$

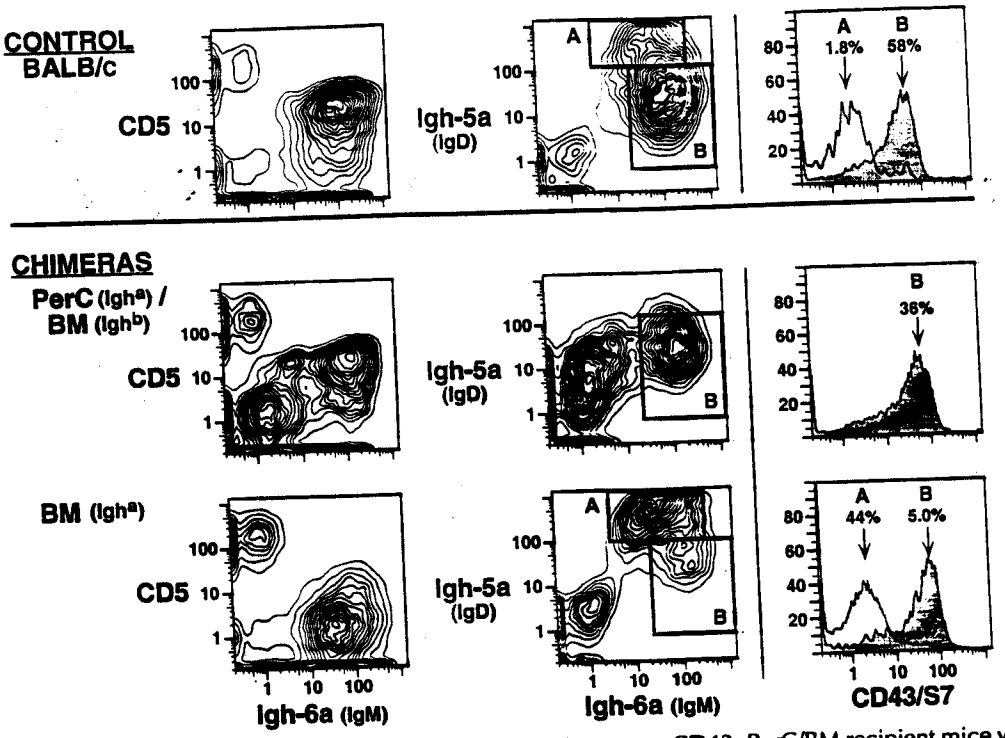

FIGURE 2. Following adoptive transfers, donor peritoneal B-1 cells express CD43. PerC/BM recipient mice were constructed by transferring BALB/c (Igh-C^a allotype) peritoneal cells along with BAB/25 (Igh-C^b allotype) BM into lethally irradiated BAB/25 recipients. BM recipient mice were constructed by transferring BALB/c BM in the absence of peritoneal cells into lethally irradiated BAB/25 recipients. Control BALB/c mice were unmanipulated. Three months following reconstitution, peritoneal cells were stained for Igh-6a(FI), Igh-5a(TR) and CD5(APC) or Igh-6a(FI), Igh-5a(APC) and S7(PE). PI was used to gate out dead cells in either the PE or TR channels. The left-hand panels show the Igh-6a vs CD5 profiles of the three mice. B-1 and conventional B cells were gated according to their IgM (Igh-6a) and IgD (Igh-5a) phenotypes (*middle panels*). Histograms in the right-hand panels show the expression levels of CD43/S7 of the gated populations. Shaded histograms (*B*) represent gated B-1 cell populations (Igh-6a^{bright}, Igh-5a^{dull}). Open histograms (*A*) represent conventional B cell populations (Igh-6a^{dull}, Igh-5a^{bright}). The percentage of peritoneal lymphocytes that each population represents is given above each histogram. Analysis based upon 30,000 cells collected.

by 6 mo of age (Table I). In addition, there is a decrease in the ratio of B-1a to B-1b cells as mice age. The frequency of B-1a cells drops from about 80% in 1-mo-old BALB/c and BAB/25 mice to 50 to 60% at 6 mo of age.

Analysis of the PerC B-1 population from mice of different strains also reveals variation of the frequency of CD43/S7$^-$ and CD43/S7$^+$ B-1 cells. When compared to age-matched BAB/25 mice housed in the same facility, both C57BL/6 and CBA/Ca have higher frequencies of CD43/S7$^-$ B-1 cells with CBA/Ca mice having the highest frequency (14% of B-1 cells) of all three strains. Consistent with earlier reports (7), CBA/Ca and C57BL/6 have fewer total B-1 cells than do BALB/c or BAB/25 mice of the same age. The ratio of B-1a to B-1b cells is also lower for both the CBA/Ca and C57BL/6 mice.

CD43/S7 phenotype of peritoneal B-1 cells in PerC/BM chimeric mice is indistinguishable from that of peritoneal B-1 cells of normal mice

We have shown previously that after adoptive transfer, B-1 cells undergo self replenishment and maintain the phenotypic characteristics of the donor population for up to 5 mo. Using chimeric mice in which B-1 and conventional B cells are clearly distinguishable by differences in Ig heavy chain allotype (7, 11, 34), we determined the expression of CD43 on self-replenished B-1 cells after transfer. PerC/BM chimeras were constructed by adoptively transferring peritoneal cells from BALB/c (a allotype) and BM from allotype congenic BAB/25 (b allotype) mice into lethally irradiated BAB/25 recipients. Allotype-specific Abs were then used to identify B-1 cells that originated from the peritoneal donor. As we have previously shown, the transferred peritoneal cells give rise to normal numbers of B-1a and B-1b cells in the PerC of recipient mice (Fig. 2, left set of panels), whereas the transferred BM cells reconstituted mostly conventional B cells (and the rest of the hematopoietic system) (data not shown).

Peritoneal B-1 cells from both PerC/BM chimeras and normal controls are predominantly CD43/S7$^+$ and have indistinguishable patterns of expression with respect to both level and frequency. (Fig. 2; control BALB/c vs PerC/BM donor, histograms labeled *B*). In contrast, conventional B cells from

both BM recipients and normal controls are essentially all negative for CD43/S7 (histograms labeled *A*).

B-1b cells derived from adult BM are predominantly CD43/S7$^+$

Although BM contains very little progenitor activity for B-1a cells, we have recently shown that adult BM (in the absence of mature B-1 cells) can give rise to small numbers of B-1 cells (predominately B-1b) in transfer recipients, suggesting that functional progenitors for B-1b cells survive longer into adulthood than do progenitors for B-1a cells (10, 11, 21). Although no phenotypic differences between PerC- and BM-derived B-1 cells have been found, functional and repertoire differences between the two populations may exist. At all ages the percentage of CD43/S7$^-$ cells is higher for B-1b cells than B-1a cells. One possible explanation is that B-1b cells that preferentially develop from adult BM do not express CD43/S7.

To test this possibility, BALB/c BM recipients were analyzed. In agreement with our earlier studies, the donor BM predominately reconstitutes conventional B cells. It is clear from the Igh-6a vs Igh-5a profiles that fewer B-1 cells are reconstituted from BM than from the PerC (Fig. 2). On average, BM reconstitutes B-1 cells (the majority of which are B-1b) to 20% of normal (11). Strikingly, the level of expression of CD43/S7 on BM-derived B-1 cells is indistinguishable from control BALB/c peritoneal B-1 cells. Thus, the B-1(b) cells that are derived from BM, like those derived from Ig$^+$ precursors in the perC, are predominantly CD43/S7$^+$.

Splenic B-1 cells can be clearly identified in PerC/BM chimeric mice

Identification and study of splenic B-1 cells has been exceedingly difficult because of their low frequency and the lack of surface markers such as CD11b. Studies have been limited to analyses of chimeric mice in which donor-derived splenic B-1 cells can be identified by their donor allotype. This self replenishment of B-1 cells is one of their defining properties (10, 14) and distinguishes them from conventional B cells (A. B. Kantor, A. M. Stall, S. Adams, and L. A. Herzenberg, in preparation). Having shown that the peritoneal B-1 cells maintain their expression of CD43/S7 in the PerC/BM chimeras, we examined whether the donor-derived B-1 cells in the spleen showed a similar CD43/S7 expression pattern to PerC B-1 cells.

CD43 is expressed at high levels on T cells and macrophages (Fig. 3*B*). To minimize interference from background staining and enhance visualization of the exceedingly small population of splenic B-1 cells in both normal and chimeric animals, one fluorescence channel is used to gate out T cells, granulocytes, and dead cells (Dump; Fig. 3*A*). The three remaining fluorescence channels are then available to determine the phenotype of the Dump$^-$ cells.

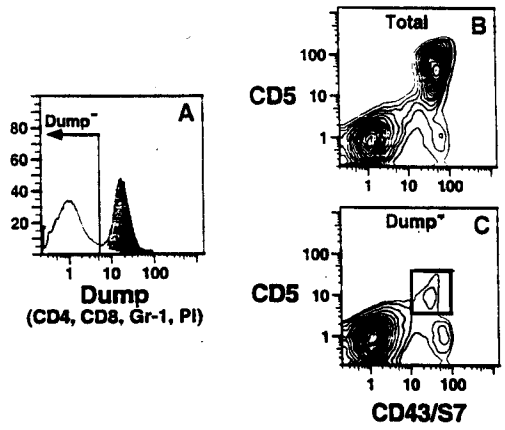

FIGURE 3. Identification of CD5$^+$, CD43$^+$ cells in spleen. Spleen cells from a 2-mo-old BAB/25 mouse were stained with S7(PE) and CD5(APC). Abs specific for CD4, CD8 and GR-1 were used as a dump to eliminate T cells and granulocytes in the TR channel. PI was monitored in this channel and a hard gate was used to exclude most of the dead cells which are the brightest cells in this channel. (*A*) A histogram of the dump (CD4, CD8, 8C5) and the arrow shows the gate used to eliminate all positive-staining cells. The right-hand panels show the CD43/S7 and CD5 phenotype of total spleen cells (*B*) and of spleen cells which are left after gating on Dump$^-$ cells (*C*). Note the population of CD43/S7^{dull}, CD5^{dull} cells which is resolved only following gating on the Dump$^-$ cells (boxed population).

Gating out Dump$^-$ cells (which account for 48% of splenocytes) unmasks a subpopulation of CD43/S7$^+$, CD5$^+$ cells (boxed population) which accounts for only 1.4% of total spleen cells and cannot be distinguished in the plot of total splenocytes (Fig. 3*C*). This small population enclosed in the box is not a contamination of T cell in the dump$^-$ gate because: a) the frequency is not reduced by using a tighter gate; b) the level of CD5 expression is characteristic of B-1 cells and not T cells; and c) the cells in this population express IgM and B220 (see Fig. 6).

Gating on the Igh-6a$^-$ (Dump$^-$) B cells allows characterization of donor B-1 cells within the chimeras (Fig. 4). Although there is some overlap in the populations because of the broad range of Igh-6a expression, as observed in the PerC of the PerC/BM chimera, splenic conventional B cells (Igh-6a$^-$) are predominantly CD23$^+$, whereas both B-1a and B-1b cells (Igh-6a$^+$) are CD23$^-$. The majority of the PerC-derived splenic B-1 cells in these chimeric mice are B-1a cells, reflecting their higher frequency in the donor population (Fig. 4, total PerC donor). In chimeric mice constructed with sorted peritoneal B-1a or B-1b cells, splenic (and peritoneal) B-1a cells are preferentially reconstituted from transferred B-1a cells (Fig. 4 sorted B-1a and B-1b donors, peritoneal data not shown). The B-1b chimeras, which received one-third the number of sorted cells as the B-1a chimera, similarly yielded smaller splenic

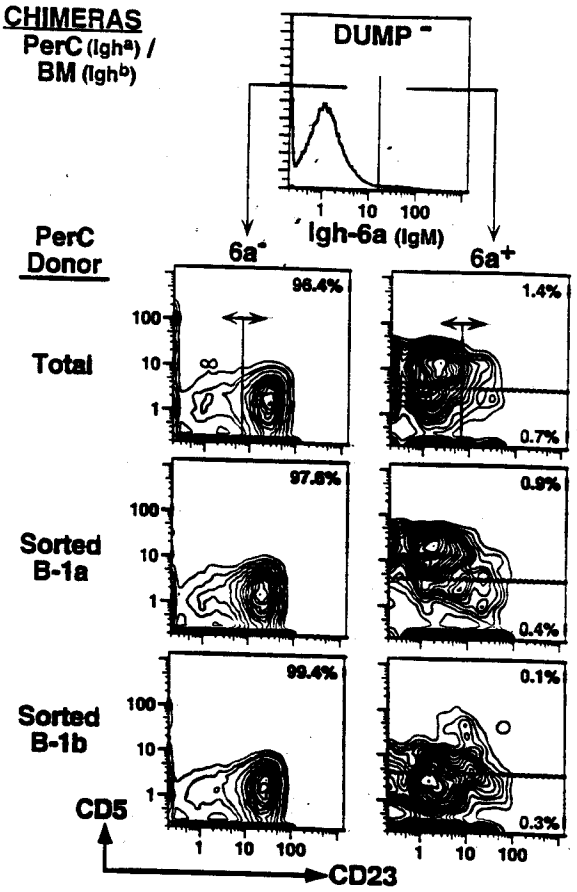

FIGURE 4. Identification of donor B-1 cells in spleen of PerC/BM chimeric mice. PerC/BM chimeric mice were constructed as described in Figure 2 except that sorted BALB/c B-1a ($CD5^+$) or B-1b ($CD5^-$) cells were also transferred into lethally irradiated BAB/25 (b allotype) recipients along with BAB/25 BM. Spleen cells from these mice were stained with Igh-6a (FI), CD23 (TR), CD5 (APC), and Dump Abs (PE) + PI (Fig. 3). Igh-6a expression of $Dump^-$ cells in the spleen is shown in the top histogram. The Igh-$6a^-$ (BM-derived conventional B cells) and Igh-$6a^+$ (PerC-derived B-1) cells were gated to show the CD23 and CD5 phenotype. The positive and negative gate is shown for CD5 (dashed line). The cutoff for CD23 is at 7, which is higher than in Figure 1, because in this analysis we used a ^{bi}anti-CD23/TR-AV stain which has a significantly higher background. The phenotype of conventional B cells (Igh-$6a^-$) in all three mice looks identical. In the mice reconstituted with sorted B-1b cells, the recipients are clearly enriched for B-1b cells. The percentages given in the contour plots are for $Dump^-$ cells.

(0.4%) populations of B-1 than the B-1a chimeras (1.3%). The Igh-C^a allotype B-1a cells which appear in the B-1b chimera most likely reflect contamination in the original sort. These analyses demonstrate that, as in the PerC (Fig. 2), the splenic B-1 cells maintain the phenotypic characteristics of the donor B-1 cells.

Splenic B-1 cells in PerC/BM chimeras are CD43/S7$^+$

The expression of CD43/S7 was analyzed on both splenic B-1a and B-1b cells from the sorted B-1 chimeras. Probability plots of B220(6B2) vs CD43/S7 are shown for $Dump^-$ cells in the left-hand panels of Figure 5. The center and right-hand panels display B220 and CD43/S7 expression of the $Dump^-$ cells, gated for Igh-$6a^+$ (PerC-derived B-1) and Igh-$6b^+$ (BM-derived conventional) B cells. It is clear that the Igh-$6a^+$ (PerC-derived B-1) and Igh-$6b^+$ (BM-derived conventional) B cells represent two phenotypically distinct populations. As a population, the PerC-derived B cells express lower levels of B220, whereas the BM-derived B cells express high levels of B220. Thus, consistent with previous studies, splenic B-1 cells, like PerC B-1 cells, express lower levels of B220/6B2 than conventional B cells (7). Similarly, the two populations express distinct levels of CD43. For the PerC-derived population the level centered at a value of 10, however, for the BM-derived population CD43 was indistinguishable from background centering at a value of 1–2. The data in Figure 5 indicate that in the transfer recipients donor B-1 (both B-1a and B-1b) cells (which are identified as both Igh-$6a^+$ and $B220^{dull}$) are, as in the PerC, CD43/S7 positive.

There is a population of $B220^{bright}$ $CD43^-$ cells in each of the Igh-$6a^+$ panels for the PerC/BM chimeras. We believe that these are most likely contaminating BM-derived cells for the following reasons: a) the frequency is increased by using less restrictive gates; b) the smaller the population of Igh-$6a^-$ cells, the higher the relative contamination (total compared with sorted B-1b); c) the phenotype of these contaminating cells is identical to conventional B cells with respect to IgD, CD5, and CD23 (data not shown); and d) we see no similar populations in the PerC in which donor B-1 cells constitute >80% of the B cells.

Analysis of BM recipients described in the last section suggests that the small population of $B220^{dull}$, $CD43/S7^+$ cells in the Igh-$6b^+$ cells are mostly likely BM-derived B-1(b) cells. Additional gating demonstrates that essentially all of the Igh-$6a^-$, $CD5^+$ spleen cells are $CD43/S7^+$ in the B-1a/BM chimera (data not shown).

Together with the in vivo studies presented in the next section, the analyses of the PerC/BM chimeras indicate that splenic B-1 cells can be readily distinguished from conventional B cells which are $B220^{bright}$ and $CD43/S7^-$.

Expression of CD43/S7 phenotypically distinguishes B-1 cells from other CD23$^-$ cells

Because analyses of the chimeric mice indicated that both splenic B-1a and B-1b cells are $B220^{dull}$ and $CD43/S7^+$, we compared the phenotype of the corresponding population to total spleen cells in normal mice (Fig. 6). The majority of cells within the $B220^{dull}$, $CD43/S7^+$ gate have a phenotype indistinguishable from the peritoneal B-1 cells

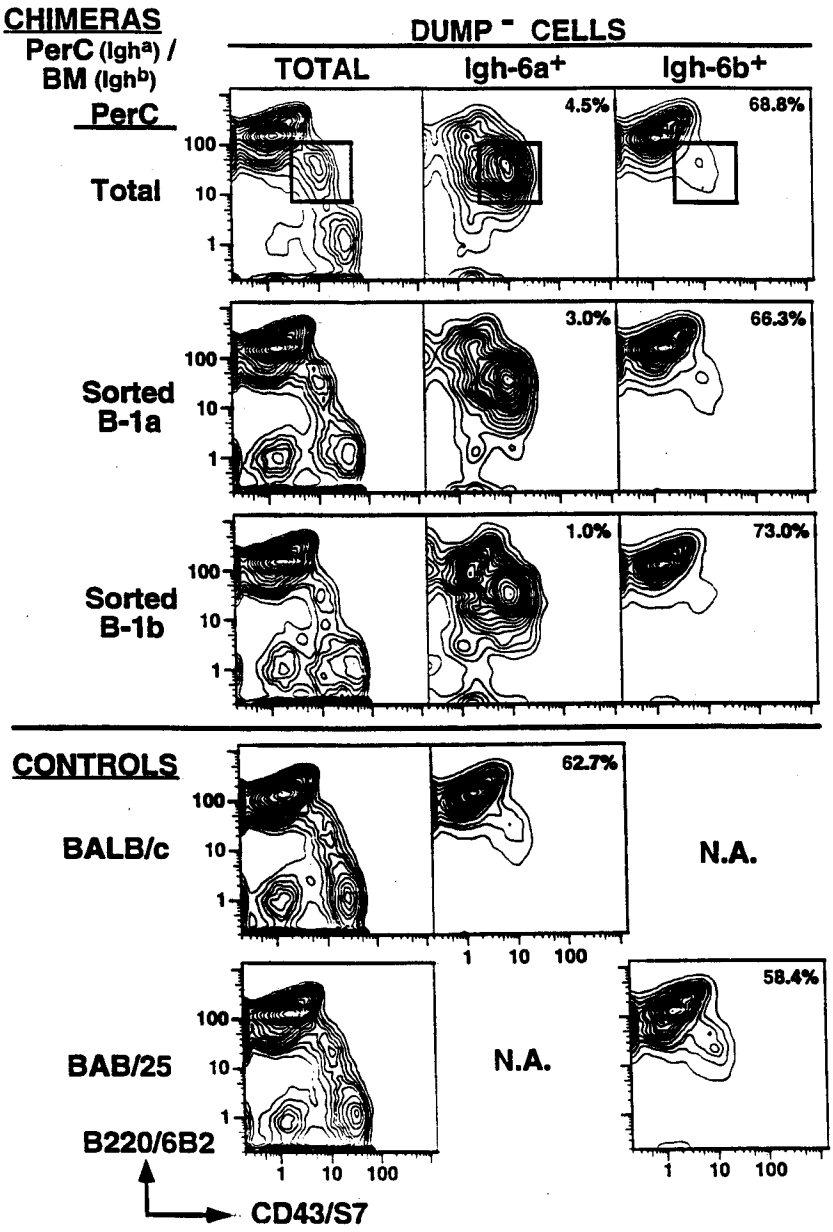

FIGURE 5. Donor B-1 cells in spleen of PerC/BM chimeras express CD43. Sorted PerC/BM chimeric mice were constructed as described in Figure 4. Spleen cells were stained with anti-B220/6B2 (TR), anti-CD43/S7 (Fl), Dump (PE) and anti-Igh-6a or anti-Igh-6b (APC). The B220/6B2, CD43/S7 phenotype of total Dump$^-$ spleen cells from total PerC/BM, B-1a/BM, B-1b/BM chimeras and BALB/c and BAB/25 controls are shown in the left-hand panels. The center panels show the B220/6B2, CD43/S7 phenotype of only those cells which are Igh-6a$^+$ (B-1 cells) gated as shown in Figure 4. The phenotype of the Igh-6b$^+$ (conventional B) cells are shown in the right hand panels. The percentages of Dump$^-$ cells within each gated population are shown in the upper right-hand corner of each panel.

and splenic B-1 cells identified in the PerC/BM chimeras. These cells are: IgD^{dull}, confirming that they are not follicular B cells; mostly IgM^{bright}, again consistent with the B-1 phenotype; and CD5^{dull}, characteristically duller for CD5 than splenic T cells. The isotype control, anti-CD4, reveals no staining. The small population of B220^{dull} CD43/S7$^+$, IgM$^-$ Dump$^-$ cells observed in the splenic profiles of Figure 6 most likely include 1) macrophages, which are CD43/S7$^+$ and highly autofluorescent, 2) pre/pro B cells, which are also B220^{dull}, CD43/S7$^+$ (4), and 3) classic plasma cells which are CD43/S7$^+$ but lack significant surface Ig (1).

Because both peritoneal and splenic B-1 cells are B220^{dull} (7), the fact that no CD5$^+$ B cells were seen in the B220^{dull} CD43$^-$ population (data not shown) suggests that within the limits of our analyses all splenic B-1a (and presumably B-1b) cells are included within the B220^{dull} CD43$^+$ population. These results, consistent with the adoptive transfer experiments, demonstrate that expression of CD43/S7 provides a means by which splenic B-1 and follicular B cells can be distinguished.

Although the B220^{dull} CD43$^+$ population may include all B-1 cells, it could potentially include other populations such as MZ or IB cells. To eliminate these potential sources of contamination, we utilized an alternative gating approach as shown in Figure 7. Gating on IgM^{bright} cells discriminates mature B cells from contaminating macrophages, pro/pre-B and Ig$^-$ plasma cells. This was confirmed by cytological analysis of sorted IgM^{bright}, CD23$^-$, CD43/S7$^+$ which revealed that <1% of the cells exhibited characteristic plasma cell morphology, including high cytoplasmic Ig and low nuclear/cytoplasm ratio (data not

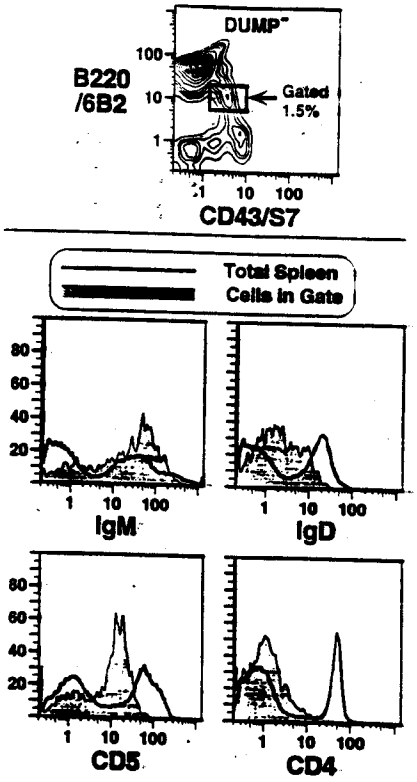

FIGURE 6. CD43$^+$ B cells in spleen display the characteristic B-1 cell phenotype. Spleen cells from a normal BALB/c mouse were stained for B220/6B2 (TR), CD43/S7 (FI), Dump (PE)+PI and IgM, IgD, CD5, or CD4 (APC). The B220/6B2, CD43/S7 phenotype of total Dump$^-$ cells is shown in the top panel. The B220/6B2^{dull}, CD43/S7^{dull} cells are gated as shown. These cells account for 1.5% of the total Dump$^-$ spleen cells. Shaded histograms in the four bottom panels show the phenotype of the gated cells; open histograms show the phenotype of total spleen cells (no dump gate). The *y*-axis is a normalized scale for each histogram.

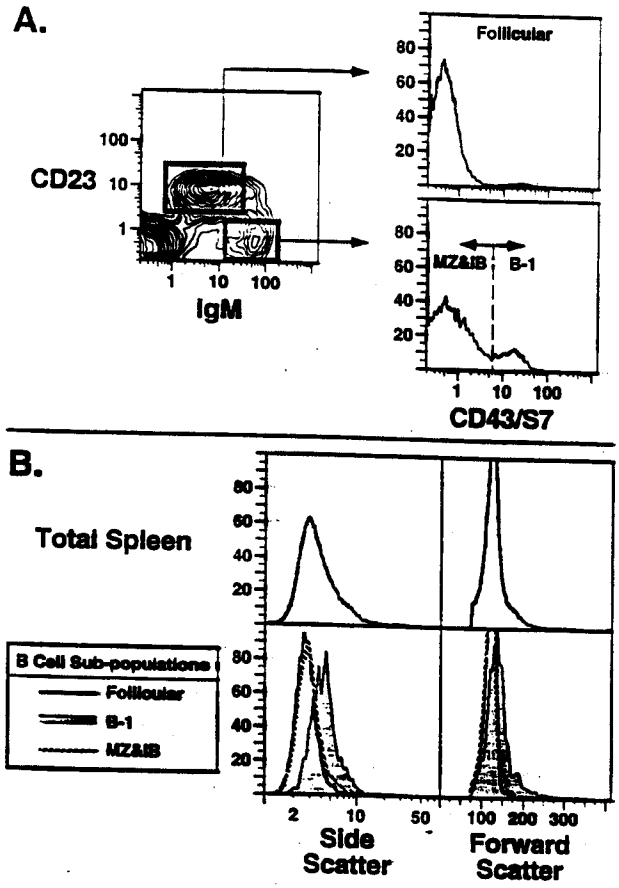

FIGURE 7. Phenotypic identification of splenic B cell subpopulations. (*A*) Spleen cells from 1 to 2-mo-old BAB/25 mice were stained for CD23 (TR), IgM (FI) and CD43/S7 (PE). The CD23, IgM phenotype of total spleen cells is shown in the left-hand panel. Both MZ&IB cells and B-1 cells are found within the IgM^{bright}, CD23$^-$ gate, but these two populations can be distinguished by their CD43/S7 phenotype. The MZ&IB, which make up the majority of this population, are IgM^{bright}, CD23$^-$, CD43/S7$^-$. The B-1 cells, however, are IgM^{bright}, CD23$^-$, CD43/S7$^-$. (*B*) The size, as defined by forward and side scatter, is shown for the B cell subpopulations defined in (*A*). The side and forward scatter profile of total spleen cells (*upper histograms*), and of follicular, MZ&IB and B-1 cells (*lower histograms*) is shown. The *y*-axis is a normalized scale for each histogram. Follicular, MZ&IB, and B-1 cells comprise approximately 85%, 12% and 3% of the total B cells respectively (Table II).

shown). Further gating on CD23$^-$ cells eliminates conventional follicular B cells (23, 35). The analysis is thus restricted to a small population (typically <15 to 20%) of splenic B cells, which includes B-1, MZ and IB cells. In addition to the lack of CD23 expression, MZ and IB cells share many phenotypic characteristics of B-1 cells, including high levels of surface IgM and low levels of surface IgD. This has made it difficult to distinguish between these two populations. Because previous studies have suggested that both MZ (23) and BM IB (4) cells lack CD43/S7 expression and our studies show that splenic B-1 cells express substantial levels of CD43/S7, we used the expression of this Ag to distinguish B-1 and MZ (and IB) cells in the spleen. The staining combination used in Figures 7–9 has the additional advantage that it uses a PE-conjugate of S7, which has a lower background and a brighter signal than the biotin/TR-avidin stain used in Figures 4–6. This reagent allows us to cleanly resolve the CD43 positive and negative populations (compare the CD43 staining in Figs. 3 and 7).

Expression of CD43/S7 divides the CD23$^-$, IgM^{bright} population in two (Fig. 7*A*). The majority of these cells (greater than 80%) were negative for CD43/S7, whereas 20% clearly express CD43. Because we gate on IgM^{bright} cells, the CD43$^+$ B-1 cell population identified here does not contain pro/pre-B cells or classical plasma cells. Based upon our studies and those of Waldschmidt et al., (23) we define splenic B-1 cells as IgM^{bright}, CD23$^-$, CD43/S7$^+$,

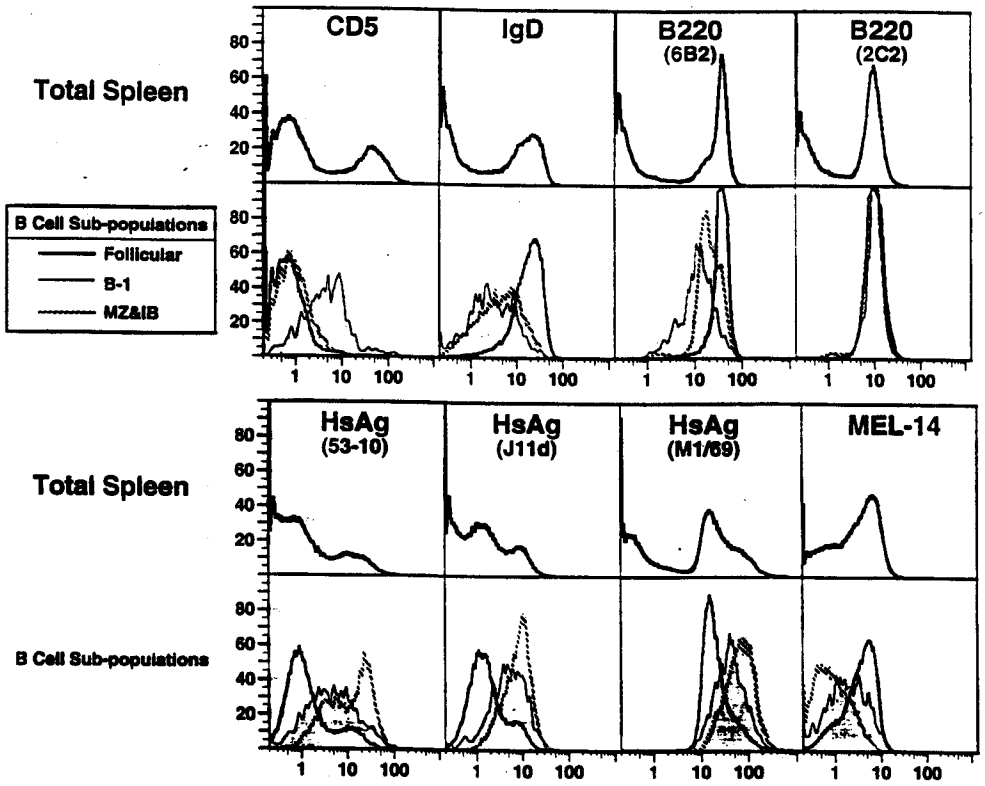

FIGURE 8. Comparative phenotypes of splenic B-1 and MZ&IB cells. Spleen cells from 1 to 2-month-old BAB/25 mice were stained for CD23 (TR), IgM (Fl), CD43/S7 (PE) and CD5 (APC). In all other samples, cells were stained for CD23 (TR), IgM (APC), CD43/S7 (PE) and Fl conjugates of anti-IgD, B220 (6B2 or 2C2), HSA (53-10, M1/69 or J11D), or MEL14. The expression of each Ag on total spleen (*upper histograms*) and on follicular, B-1 cells, and MZ&IB cells (*lower histograms*) is shown. The B cell subpopulations were gated as shown in Figure 7.

whereas MZ and IB cells are IgM^{bright}, $CD23^{-}$ and $CD43/S7^{-}$ (23, 36). From now on, we will refer to these two subpopulations collectively as the MZ&IB population.

Phenotypic differences among splenic B-1, MZ, IB, and follicular B cells

Comparison of the B-1 and MZ&IB subpopulations reveals several interesting phenotypic differences. The size of the splenic B-1 cells, as indicated by forward and side scatter, is virtually identical to that of PerC B-1 cells (data not shown); however, they are larger than MZ&IB and follicular B cells (Fig. 7*B*). The panels in Figure 8 show the expression of various B cell surface Ags on total spleen cells (*upper panels*), follicular, B-1 and MZ&IB cells (*lower panels*). As previously shown, both B-1 and MZ&IB cells express similarly low levels of IgD (23, 37, 38). Also as expected, the majority of the B-1 cells express CD5 at a level identical to PerC B-1a cells. Similarly, the ratios of B-1a to B-1b cells in the spleen and PerC seem to correlate (data not shown).

B220/6B2, an isoform of CD45, is expressed at different levels on splenic B cell subsets: B-1 cells express low levels, MZ&IB cells express intermediate levels, and follicular B cells express high levels. In contrast, expression of B220/2C2 is indistinguishable on these populations. Expression of total CD45 as recognized by MAb 30F11.1 is also indistinguishable on follicular, B-1, and MZ&IB cells (data not shown).

Splenic B-1 and MZ&IB cells differ in the level of expression of the heat stable Ag (HsAg) as detected by three different mAbs, 53-10, J11d and M1/69. These mAbs show similar relative profiles but differ with respect to the intensity of staining on B cells. With all three Abs, follicular B cells express the lowest amount of HsAg, whereas B-1 cells express intermediate levels and MZ&IB cells express the highest levels of HsAg. Interestingly, detection of HsAg by 53-10, but not J11d or M1/69, reveals a bimodal distribution of HsAg on the MZ&IB population as the mice age. At 1 mo of age, the MZ&IB cells stain brightly, as a homogeneous population (data not shown), however, by 2 mo of age (Fig. 8), a duller 53-10 staining population of MZ&IB cells appears.

Finally, MEL-14, a homing receptor which is present on all murine lymphocytes that home to peripheral lymph nodes (39, 40), is expressed at different levels on the splenic B cell subpopulations. The majority of follicular B cells express a significant level of MEL-14, whereas most of the MZ&IB show little or no expression of this Ag. B-1

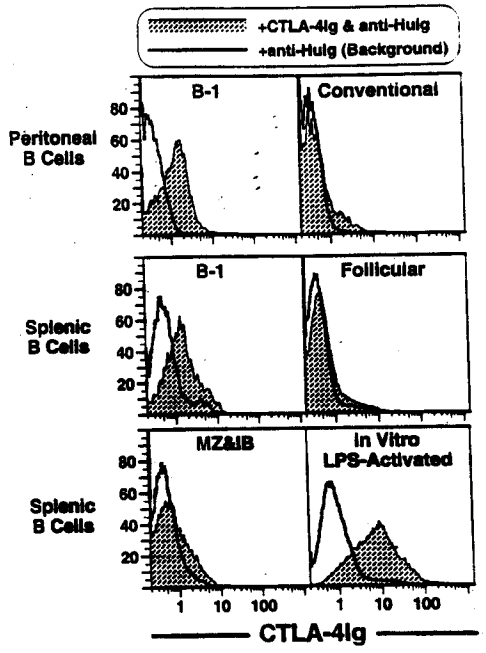

FIGURE 9. B-1 cells express low levels of CTLA-4 ligand/receptor. Spleen and peritoneal cells from 2-mo-old mice were incubated with (shaded histograms) or without (open histograms) CTLA-4Ig and then rat serum. Peritoneal cells were stained with goat anti-human IgG (Fl), IgM (APC) and IgD (TR). Spleen cells were stained with goat anti-human IgG (Fl), IgM (APC), CD23 (TR) and CD43/S7 (PE). Peritoneal B-1 cells were identified by their IgM^{bright}, IgD^{dull} phenotype, and peritoneal conventional B cells were identified by their IgM^{dull}, IgD^{bright} phenotype as in Figure 1. Splenic B cell subpopulations were identified as in Figure 7. Total spleen cells were cultured in vitro for 24 h in LPS. Cells were incubated with (shaded histogram) or without (open histogram) CTLA-4Ig, rat serum, and then stained with goat anti-human IgG(Fl) and IgM(APC). The binding of CTLA-4Ig cells is shown for total IgM^+ cells.

cells, however, express intermediate levels of MEL-14 that is different from both the follicular and MZ&IB cell populations.

Except for CD11b (Mac-1), which, as observed previously, is expressed on peritoneal but absent on splenic B-1 cells (11, 22), we found no difference between splenic and peritoneal B-1 cells in the expression levels of most of the Ags examined, including I-A, I-E, H-2K, IL-2R (CD25), CD45 isoforms (6B2, 2C2 and 30F11.1), Ly-6C, LFA-1a, ICAM-1, L-PAM, and Pgp-1 (data not shown). There is, however, a difference in expression of HsAg between peritoneal and splenic B-1 cells. Unlike splenic B-1 cells which express low levels of HsAg as detected by both M1/69 and 53-10, peritoneal B-1 cells express similar levels of M1/69, but most do not express detectable 53-10 (data not shown). This suggests that although HsAg (as detected by M1/69 and Jlld) is present on peritoneal B-1 cells, the epitope recognized by 53-10 is missing on most B-1 cells in this location (data not shown). In addition, MEL-14 appears to be expressed at slightly higher levels on peritoneal B-1 cells than on splenic B-1 cells.

The counter-receptor for both CD28 and CTLA-4, B7/BB1, was first described as an Ag that is present on the surface of activated and neoplastic human B cells, but not on resting B cells or monocytes (41, 42). Murine B-1 cells express some markers that are also induced on B cells following various activation protocols; however, they cannot be simply classified as "activated B cells" (10). To determine whether they express B7/BB1, we stained them with human CTLA-4Ig, which also recognizes the murine counter-receptor (42). As a positive control for B7 expression, total splenic lymphocytes were cultured for 24 h in LPS (Fig. 9). Although conventional B cells from PerC, and follicular and MZ&IB cells from spleen lack detectable B7/BB1 expression, B-1 cells from both locations express low levels. B7/BB1 expression on B-1 cells, however, is significantly lower than on in vitro LPS-stimulated splenic B cells.

Finally, the ability to cleanly distinguish splenic B-1 cells allows us to accurately determine the frequency of B-1 and MZ&IB cells in different ages and strains of mice (Table II). Interestingly, although the frequency (and absolute numbers) of peritoneal B-1 cells increase with age, the frequency and number of splenic B-1 cells decrease. Similar decreases are seen in the MZ&IB cell population. Three percent of the IgM^+ B cells in 1-mo-old BAB/25 spleen cells are B-1, but in 6-mo-old mice, this population decreases to 1.5%. This decrease occurs within both the B-1a and the B-1b populations. The frequency of MZ&IB cells decreases from about 13% in 1-mo-old to less than 7% in 6 mo-old mice.

Discussion

Previous studies of B lineage cells demonstrated expression of CD43 at two discrete stages of B cell development; IgM^- pro-B cells (4) and LPS-induced B cells in the terminal phases of plasma cell differentiation (1). However, similar analyses of mature IgM^+ B cells in spleen failed to reveal $CD43/S7^+$ B cells within either the $CD23^+$ or $CD23^-$ fractions (23). In this paper we present evidence that expression of the cell surface Ag CD43 (leukosialin), as identified by the mAb S7, can be used to identify splenic B cell subsets and in particular distinguishes splenic B-1 cells. Adoptive transfer experiments using chimeric mice in which B-1 and conventional B cells are identified by allotype, confirmed that both splenic B-1a and B-1b cells express similar levels of CD43/S7 but BM-derived conventional B cells lacked expression of this Ag. Furthermore, in vivo FACS analysis of splenic B cells from BAB/25, BALB/c, CBA/Ca, and C57BL/6 mice show that within the limits of our detection, virtually all splenic B-1a express CD43/S7. The present studies demonstrate that anti-CD43/S7 in conjunction with anti-IgM and anti-CD23 can be used to clearly distinguish B-1 cells

Table II. *Frequency of B-1, MZ&IB, and follicular B cells in the spleen*

Strain (age)	n	IgM$^+$ B Cells (% of total spleen)	Percent of IgM$^+$ B Cells			
			Follicular (CD23$^+$)	CD23$^-$		
				MZ&IB (CD43/S7$^-$)	B-1 (CD43/S7$^+$)	
					B-1b (CD5$^-$)	B-1a (CD5$^+$)
BAB/25 (1 mo)	6	42 (± 5.9)	79 (± 2.2)	13 (± 1.3)	0.6 (± 0.07)	2.4 (± 0.6)
BAB/25 (3 mo)	6	44 (± 1.7)	83 (± 2.3)	9.2 (± 2.1)	0.3 (± 0.1)	0.9 (± 0.3)
BAB/25 (6 mo)	5	48 (± 2.5)	87 (± 3.7)	6.5 (± 2.0)	0.3 (± 0.01)	0.9 (± 0.3)
CBA/Ca (3 mo)	5	50 (± 3.2)	79 (± 4.2)	14 (± 3.4)	0.4 (± 0.06)	1.1 (± 0.1)
C57BL/6 (3 mo)	3	56 (± 2.6)	86 (± 2.1)	9.2 (± 1.3)	0.3 (± 0.01)	1.2 (± 0.2)

from conventional B cells (including follicular, MZ and IB cells) in unstimulated adult spleen. We identify splenic B-1 cells as IgM^{bright}, CD23$^-$, CD43/S7$^+$ and subdivide them into CD5$^+$ (B-1a) and CD5$^-$ (B-1b) subsets. In contrast, MZ and IB cells are IgM^{bright}, CD23$^-$, CD43/S7$^-$, and follicular B cells are most easily defined as IgM$^+$, CD23$^+$.

Although the majority (70 to 95% in our studies) of peritoneal B-1 cells express CD43/S7, in every strain analyzed we also observed a distinct population of CD43/S7$^-$ peritoneal B-1 cells, particularly within the B-1b cells which increased with age. This increase was not due to the de novo generation of new CD43/S7$^-$ B-1b cells from BM, because BM-derived B-1(b) cells express identical levels of CD43/S7 compared with naturally arising B-1 cells (Fig. 2). In addition to age, the frequency varies with strain (CBA/Ca vs BAB) and possibly environment (BALB/c (Stanford) vs BAB (Columbia) (Table I).

Splenic B-1 cells are phenotypically distinct from other splenic B cell subsets but are similar to peritoneal B-1 cells

Earlier studies demonstrated that B-1 cells found in spleen following reconstitution with peritoneal B-1 cells, lacked CD11b (Mac-1), but were otherwise similar to their peritoneal counterparts in the expression of IgM, IgD and B220 (11, 22). Our studies confirm and extend these earlier findings and show that in normal mice, splenic, and peritoneal B-1 cells are, with the exception of CD11b (Mac-1), HsAg (53-10), and MEL-14 expression and to some extent the frequency of CD43/S7$^+$ cells, indistinguishable in phenotype. Expression levels of I-A, I-E, H-2K, CD45 isoforms (6B2, 2C2 and 30F11), Ly-6C, LFA-1a, ICAM-1, L-PAM, Pgp-1, IgD, and IgM appear to be identical on B-1 cells in these two locations.

The expression of CD43/S7 has enabled us to further distinguish splenic B-1(b) from MZ&IB cells that express similar levels of IgM, IgD and CD23 (23, 36, 37). Expression levels of HsAg, B220/6B2, and MEL-14 differ on these splenic B cell populations (Fig. 8). B-1 cells express intermediate levels of HsAg, whereas follicular B cells express low levels of HsAg. In young mice (<1 mo of age), most of the MZ&IB fraction of splenic B cells express high levels of HsAg (data not shown) and are likely IB cells as described by Allman et al., as HsAg^{high}, B220^{low} (38). However, in older mice (2 to 6 mo), HsAg expression (as detected by 53-10) on MZ&IB cells becomes bimodal, with a new population of cells expressing intermediate levels of HsAg (similar to B-1 cell levels). It is possible that these are newly arising MZ B cells. Although HsAg seems to be present in some form on all B cells, Kay et al. (43) have shown that different cell types have different glycosylation patterns of HsAg, which may account for differences seen with various anti-HsAg mAbs. Linton et al. (44) describe primary Ab-forming cells to be among the brightest 20 to 40% of HsAg-expressing B cells and secondary B cells to be among the lowest 10%. Based upon our studies, cells fractionated by either criterion will include a mixture of follicular, B-1, and MZ&IB cells. In any case, care must be used when comparing data from studies using different anti-HsAg mAbs.

Expression of the B cell isoform of CD45, B220, (as measured by RA3-6B2) is also distinctly lower on splenic and peritoneal B-1 cells compared with either MZ&IB (twofold lower) or follicular (5 to 10-fold lower) B cells. Also consistent with the phenotype of peritoneal B-1 cells (7), expression of CD45/B220 as measured using a different mAb, RA3-2C2 (45), is identical on all three B cell populations (Fig. 8). Similarly, using a pan-CD45 mAb, 30F11 (28), we detect no difference in the total expression

of CD45 isoforms on B-1, MZ&IB or follicular B cells (data not shown). This variance in the expression of 6B2 and 2C2 demonstrates a clear difference in the pattern of CD45 isoforms expressed by each of the B cell subsets.

We have shown that the CTLA-4 receptor/ligand is constitutively expressed on both splenic and peritoneal B-1 cells at low levels, but not on conventional B cells (Fig. 9). The expression of B7/BB1 and the manner in which it relates to various B cell developmental and activation state(s) require further investigation. However, by itself, the expression of the CTLA-4 receptor/ligand on B-1 cells suggests that this population is capable of stimulatory interactions with T cells via the CD28 molecule.

Are B-1 cells activated?

From these studies we have a much clearer phenotype of both splenic and peritoneal B-1 cells. It has been noted that B-1 cells share some phenotypic characteristics with B cells activated following LPS or anti-IgM stimulation (46). These include lower expression of B220/6B2 and IgD and the absence of CD23. This has led some investigators to propose that the B-1 phenotype is induced by antigenic (TI-2) stimulation and is unrelated to the lineage origin of the B cells (46, 47).

The activation state of B cells is characterized by several criteria, including secretion and proliferation as well as phenotype. Based upon the first two criteria, most peritoneal and splenic B-1 cells are not activated, because in an unimmunized animal, >95% of the CD43/S7$^+$ splenic B-1 cells are not secreting as measured by ELISA-spot assay (Wells et al., manuscript in preparation) and they are not actively proliferating (17). However, from the data we can state that peritoneal and splenic B-1 cells have a unique phenotype which is distinct from that typically associated with either activated or resting B cells for the following reasons: 1) compared to B-1 cells, HsAg expression (as detected by the mAb 53-10) is induced to significantly higher levels on B cells by both in vitro activation with the polyclonal B cell mitogen, LPS (48) and in vivo activation with Ag (Wells et al., manuscript in preparation); 2) recent studies in our laboratory demonstrate that Ag-specific activation, which results in a high level of Ab secretion by B-1 cells, also induces changes in expression levels of several surface Ags including HsAg, 53-10, B220/6B2, B220/2C2, Il-2R and CD5, but does not increase expression of CD43 (Wells et al., manuscript in preparation); 3) Although IL-2R is normally up-regulated following activation, B-1 cells show little increase in the expression of IL-2R (CD25) compared with conventional B cells (data not shown); 4) CTLA-4 receptor/ligand expression on total splenic B cells following in vitro LPS-stimulation is significantly higher than on the native B-1 population (Fig. 9). The level of CTLA-4 receptor/ligand on in vivo activated B cells is unknown.

Although the data presented here support the concept that B-1 cells are in a more "active state" than resting conventional (IgD^{bright}) B cells, it is clear that they differ from both resting B cells and those activated by Ag or LPS stimulation. B-1 cells also differ in phenotype from both MZ and IB cells. It may be most accurate to describe B-1 cells as being in a primed state. It is not possible from these studies to determine whether this state is induced by constant (antigenic) stimulation, or whether it represents a stable phenotype of a discrete B cell lineage; however, these two hypotheses are not mutually exclusive. Although the majority of studies support the hypothesis that B-1 cells represent a separate developmental lineage, it is still possible that antigenic stimulation may be required for the induction of some or most of the phenotypic characteristics associated with this population. This antigenic event could involve the initial selection of this population during fetal and neonatal development or its expansion through constant exposure to environmental Ags. Our studies provide the basis for identifying and isolating splenic B-1 cells which allows us to directly address these questions in in vivo systems. We are currently investigating the activation of B-1 cells following Ag-specific stimulation.

It is unclear what role the constitutive expression of CD43 may play in the function of B-1 cells. Gulley et al. (1) showed that in vitro culture of splenic B cells with LPS can induce high levels of CD43/S7; however, this only occurred during the terminal phases of B cell differentiation to Ig-secreting plasma cells. Given studies indicating that CD43 is important in T cell proliferation (5), the expression of CD43 on B-1 cells may be important for the self-replenishing characteristic unique to this population. Alternatively, it may indicate that "resting" B-1 cells, unlike conventional B cells, are primed for antigenic stimulation. In either case, it is unclear why the frequency of CD43/S7$^-$ B-1 cells decreases with age and/or changes in environment. If the expression of CD43/S7 does mark B-1 cells in a specific activation state, it is certainly possible that either age or environment could play a role in the frequency of B-1 cells in such a state.

Acknowledgments

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