Spontaneous Follicular Exclusion of SHP1-deficient B Cells Is Conditional on the Presence of Competitor Wild-type B Cells

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Summary

Engagement of antigen receptors on mature B lymphocytes is known to block cell entry into lymphoid follicles and promote accumulation in T cell zones, yet the molecular basis for this change in cell distribution is not understood. Previous studies have shown that follicular exclusion requires a threshold level of antigen receptor engagement combined with occupancy of follicles by B cells without equivalent receptor engagement. The possibility has been raised that follicular composition affects B cell positioning by altering the amount of available antigen and the degree of receptor occupancy. Here we show that follicular composition affects migration of mature B cells under conditions that are independent of antigen receptor occupancy. B cells deficient in the negative regulatory protein tyrosine phosphatase, SHP1, which have elevated intracellular signaling by the B cell receptor, are shown to accumulate in the T zone in the absence of their specific antigen. Follicular exclusion of SHP1–deficient B cells was found to be conditional on the presence of excess B cells that lack elevated intracellular signaling, and was not due to a failure of SHP-1–deficient cells to mature and express the follicle-homing chemokine receptor Burkitt's lymphoma receptor 1. These findings strongly suggest that signals that are negatively regulated by SHP1 promote B cell localization in T cell zones by reducing competitiveness for follicular entry, and provide further evidence that follicular composition influences the positioning of antigen-engaged B cells.

Mature recirculating B cells that have entered secondary lymphoid tissues pass rapidly through the outer T cell zone and migrate into B cell follicles (1). In contrast, mature B cells that have bound antigen are blocked from migrating into lymphoid follicles and, in most cases, take up temporary residence in the outer T cell zone (2, 3). B cells that have encountered a foreign antigen may present MHC–peptide complexes to antigen-specific T cells and be induced to differentiate into antibody-secreting cells or into germinal center cells. Studies in Ig-transgenic models have established that autoantigen-binding B cells can also be excluded from follicles and retained in the outer T zone (4, 5). In this case, T cell help may not be available due to T cell tolerance mechanisms and the autoreactive B cells undergo rapid cell death. Together, these studies indicate that retention of B cells in the outer T zone may be important both for promoting immune responses to foreign antigens and for purging cells with specificity for self-antigens.

The mechanisms controlling whether a B cell migrates into a follicle or localizes in the T zone are poorly defined. Immature B cells that have left the bone marrow and entered the spleen do not migrate directly into lymphoid follicles but appear both in the red pulp and T zone (6). This may reflect a developmentally regulated property such as the absence of the orphan chemokine receptor, Burkitt's lymphoma receptor 1 (BLR1), on immature B cells (reference 7 and this manuscript). BLR1 is primarily expressed on mature B cells and is necessary for mature B cell homing into splenic follicles (8).

In B cells that have matured to express BLR1, additional factors regulate localization in outer T zones versus follicles, and these have been examined in most detail in the anti–hen egg lysozyme (HEL)[1] Ig transgenic/HEL transgenic model (9). Based on studies in this system, two models of follicular exclusion have been proposed: a competition-dependent model (4) and a cell-intrinsic model (10).

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[1]*Abbreviations used in this paper:* BLR1, Burkitt's lymphoma receptor 1; CFSE, carboxyfluorescein diacetate succinimidyl ester; HEL, hen egg lysozyme; MBP, mannose-binding protein; *me^v*, motheaten viable; Rag, recombination-activating gene.

In the competition-dependent model, B cell antigen receptor engagement by soluble HEL is necessary but not sufficient to mediate follicular exclusion, and competitor B cells that are not binding equivalent amounts of HEL antigen must also be present (4, 11). In the cell-intrinsic model, antigen receptor engagement alone is sufficient to mediate follicular exclusion (10, 12). A criticism of the studies indicating a role for competitor B cells has been that the experiments that altered the amount of competition, by changing the total frequency of HEL-binding cells, also may have altered the amount of HEL antigen. Although differences in antigen concentration or receptor occupancy have not been reproducibly detected in mice with different numbers of HEL-binding B cells (12–14), it remains possible that there are local differences in the way HEL antigen is presented within the secondary lymphoid tissues. Distinguishing between these models is important for understanding how autoreactive B cells are regulated in the periphery and for considering whether the increased frequency of autoimmunity observed in immunodeficient individuals could be a consequence of insufficient interclonal competition (15).

To explore whether mature B cells influence each other's positioning in a system that is independent of possible local effects on antigen presentation, we have examined the localization of SHP1-deficient B cells in mice containing or lacking wild-type B cells. SHP1 is an SH2-containing cytosolic protein tyrosine phosphatase (PTPase) that is widely expressed in the hematopoietic system (16). A negative regulatory function for this PTPase in B lymphocyte antigen receptor signaling was identified by studying B cells from motheaten viable (me^v) mice, which carry a mutation in SHP1 that reduces activity to 20% of wild-type levels (17–19). In the course of studies on Ig-transgenic me^v mice, it was observed that many SHP1-deficient B cells developing in the absence of antigen downregulated their surface IgM, increased MHC class II expression, and had twofold lower CD21 as they matured to an IgD^{hi} conventional B cell phenotype (19). This altered pattern of receptor expression mirrors that of wild-type Ig-transgenic B cells developing in the presence of the weak self-antigen soluble HEL (13, 19), in which continued B cell receptor engagement and chronic calcium and extracellular signal regulated kinase (ERK) signals occur (20). These observations suggest that SHP1-deficient B cells have an elevated level of basal antigen receptor signaling in the absence of antigen (21). In this report we show that SHP1 regulates the signaling pathways that mediate exclusion of mature B cells from lymphoid follicles. We also find that positioning of SHP1-deficient B cells is influenced by the presence or absence of wild-type B cells.

Materials and Methods

Mice. C57BL/6 (B6) Ig-transgenic mice were of the MD4 line, which carries transgenes encoding IgM^a and IgD^a heavy chains and a light chain that pair to form a high affinity anti-HEL specificity (9). B6 HEL-transgenic mice were of the ML5 line, which carries a transgene encoding HEL under the metallothionein promoter, and contained HEL at ~1 nM in serum (9). B6 me^v/ + mice (Jackson Laboratories, Bar Harbor, ME) were mated with B6 MD4 Ig-transgenic mice and with bcl2-22–transgenic mice (22; more than six generations backcross to B6). me^v/+ Ig/bcl2 double transgenic mice were then mated with me^v/+ nontransgenic littermates. B6 recombination-activating gene (Rag)1$^{-/-}$ mice (23) and B6 $\mu^{-/-}$ mice (24) were obtained from Jackson Laboratories.

Chimeric Mice. Lymphoid tissues were isolated as previously described (9). me^v/me^v Ig-transgenic, me^v/me^v Ig/bcl2 double transgenic, and control donors were killed at 4–7 wk of age and bone marrow was mixed with nontransgenic wild-type, Rag1$^{-/-}$, or $\mu^{-/-}$ bone marrow at the ratio stated in the Results section and 3–5 × 10^6 cells were injected into the lateral tail vein of B6 recipients that had been lethally irradiated with two doses of 450 rads X-irradiation separated by 3 h. The animals received antibiotics (polymixin B, 110 mg/liter, and neomycin 1.1 g/liter) in the drinking water for the whole 5-8–wk reconstitution period until analysis. After reconstitution, animals were killed, the spleen removed, three fourths of the organ was frozen in OCT compound (Miles Inc., Elkhart, IN) for sectioning. Cell suspensions were prepared from the remainder, counted, and analyzed by flow cytometry.

Adoptive Transfers. Donor cells isolated from the spleen of nontransgenic B6 mice were labeled with 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) as described (25). Bone marrow chimeric mice 7–8 wk after reconstitution were injected in the lateral tail vein with 0.3-ml aliquots of labeled spleen cells containing 10^7 B cells. After 1 d, the spleen was removed and used for flow cytometry and microscopy.

Preparation of Anti-BLR1 Antiserum. Sequence encoding amino acids 20–57 of mouse BLR1 (26) was isolated by reverse transcriptase PCR and introduced into the pGEX-2T (27) and pMAL-p2 (New England Biolabs, Beverly, MA) vectors. Glutathione-S-transferase (GST) and mannose-binding protein (MBP) fusion proteins were affinity purified and the GST fusion was used for immunization of rabbits following standard procedures (Josman Laboratories, Napa, CA). Serum from one rabbit showed reactivity with the MBP–BLR1 fusion protein by Western blotting and BLR1-specific antibodies were affinity purified by passage over a column of MBP–BLR1 covalently coupled to Sepharose CL4B (Pharmacia, Piscataway, NJ). Staining of cells with the antiserum was revealed with goat anti–rabbit FITC (Caltag, Burlingame, CA). The antiserum was found to specifically stain BLR1, but not vector control, transfected 293 cells (data not shown).

Immunohistochemistry and Immunofluorescence Microscopy. Cryostat sections (7 mm) were fixed and stained as previously described (14). In immunohistochemistry, HEL-binding cells were detected by incubating with HyHEL9-biotin followed by avidin-conjugated alkaline phosphatase (Sigma Chemical Co., St. Louis, MO); mAbs specific for B220 (6B2), or CD4 and CD8 (Caltag) were rat IgG antibodies and were detected with goat anti-rat–conjugated horseradish peroxidase (Southern Biotechnical Associates, Birmingham, AL). Enzyme reactions were developed with conventional substrates for peroxidases (diaminobenzidine/H$_2$O$_2$) and alkaline phosphatase (FAST RED/Napthol AS-MX). Sections were counterstained in hematoxylin and mounted in crystal mount (Biomeda Corp., Foster City, CA). For immunofluorescence microscopy, HEL-binding cells were detected by incubating with HyHEL9-biotin followed by streptavidin-conjugated
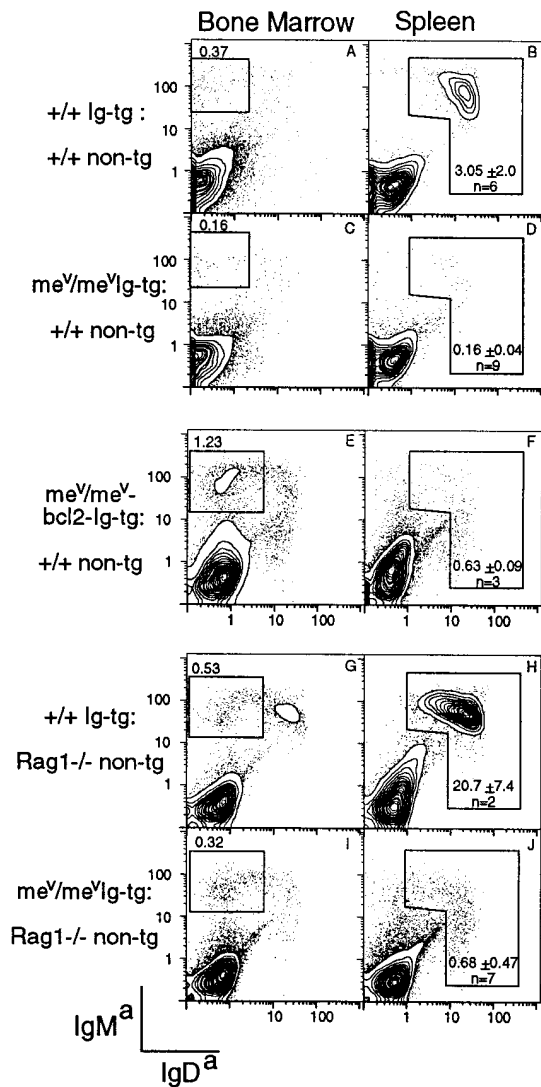
Figure 1. Frequency of immature and mature me^v/me^v or $+/+$ Ig-transgenic B cells in bone marrow chimeric mice containing and lacking wild-type nontransgenic B cells. (*A–J*) Two-color flow cytometric analysis of bone marrow and spleen cell suspensions to detect the transgenic a alleles of IgM and IgD. B cells derived from nontransgenic bone marrow express the b alleles of IgM and IgD and are not stained. The 20:80 bone marrow mixture used to reconstitute each mouse is shown to the left of the flow cytometric profiles. Adjacent panels show staining of bone marrow and spleen cells from a single bone marrow chimeric animal. Numbers in each panel refer to the percent of total cells within the gated region. The bone marrow data is representative of at least two animals of each type. Numbers shown in the right panels correspond to the mean ($+/-$ standard deviation) percentage of cells for several (*n*) animals.

Cy-3 (Jackson Immunoresearch, West Grove, PA) and marginal metallophilic macrophages were identified with MOMA-1 (28) followed by goat anti-rat–conjugated aminomethylcoumarin (Jackson Immunoresearch). Sections were mounted in a 50% glycerol/PBS mixture and viewed with a fluorescence microscope (DMRL; Leica, Wetzlar, Germany). Images were acquired on a video camera (Optronics MDEI850 cooled CCD, Optronics Engineering, Goleta, CA) and were processed with Photoshop software (Adobe Systems Inc., Mountain View, CA).

Results

B Cells with Exaggerated Signaling Due to Deficiency of SHP1 Are Spontaneously Excluded from Lymphoid Follicles. Normal B cell development is inhibited in me^v mice by trans effects of dysregulated myeloid cells (29–31). However, when bone marrow from me^v/me^v mice and from wild-type mice is mixed in a 20:80 ratio and used to reconstitute lethally irradiated wild-type recipients, development of immature B cells is restored and small numbers of mature SHP1-deficient B cells are detectable in the periphery (19). To test the follicular homing properties of SHP1-deficient B cells, mixed bone marrow chimeric mice were constructed with a 20:80 mixture of bone marrow from me^v/me^v Ig-transgenic mice and wild-type nontransgenic mice. Using bone marrow from SHP1-deficient mice carrying the anti-HEL Ig transgenes allowed the SHP1-deficient B cells to be distinguished from the wild-type B cells. In preliminary experiments, we observed that the me^v/me^v Ig-transgenic bone marrow contributed <1% of the spleen cells in these animals (Fig. 1, *C* and *D*), whereas in mice reconstituted with 20% wild-type Ig-transgenic bone marrow, 2–8% of spleen cells were Ig transgenic (Fig. 1, *A* and *B*). To generate control mice producing a comparable number of wild-type Ig-transgenic cells to the me^v chimeras, mixed chimeras were constructed with 5% Ig-transgenic and 95% nontransgenic bone marrow. After allowing 6 wk for reconstitution of the hematopoietic system, spleens were isolated from the chimeric animals, a small fraction was stained and examined by flow cytometry to measure the frequency of HEL-binding cells, and the remainder was used to prepare sections for immunohistochemistry.

Wild-type B cells carrying the anti-HEL specificity and developing in mice lacking HEL were able to migrate into lymphoid follicles and become evenly dispersed among the diverse population of nontransgenic B cells (Fig. 2 *A*). In contrast, SHP1-deficient B cells did not become evenly dispersed through follicles, but were distributed predominantly in the T cell zone and marginal zone bridging channels (Fig. 2 *B*). The marked retention of SHP1-deficient B cells in the T cell zone was also clearly seen in sections stained to detect T cells and HEL-binding cells (Fig. 2 *D*) and enumeration of the HEL-binding B cells within splenic white pulp cords established that 60% of SHP1-deficient B cells localized in regions costained for CD4 and CD8 compared with only 10% of wild-type cells (Fig. 3). Anti-HEL antibody secreting plasma cells were also detected in the red pulp of me^v/me^v–wild-type chimeric animals, but these were readily distinguished from HEL-binding B cells by their larger size and very intense cytoplasmic staining with HEL (data not shown). In some of the chimeras peanut agglutinin (PNA)-positive germinal centers were identified within splenic follicles (Fig. 2 *F*) and SHP1-deficient HEL-binding B cells were sometimes localized within these germinal centers (Fig. 2 *E*). However, even in these cases, the me^v B cells remained excluded from the surrounding mantle of resting B cells.
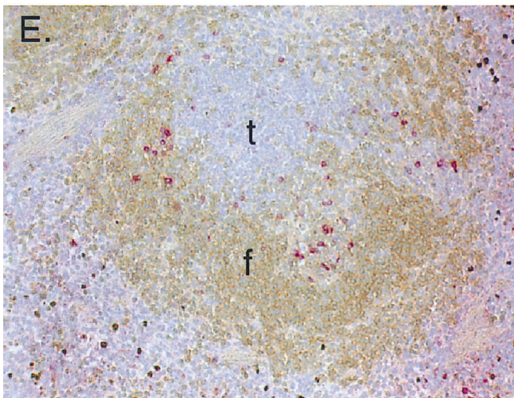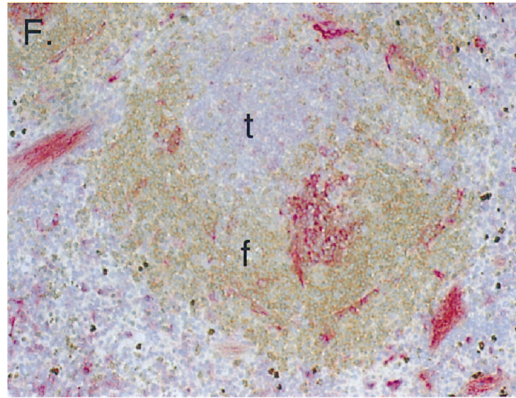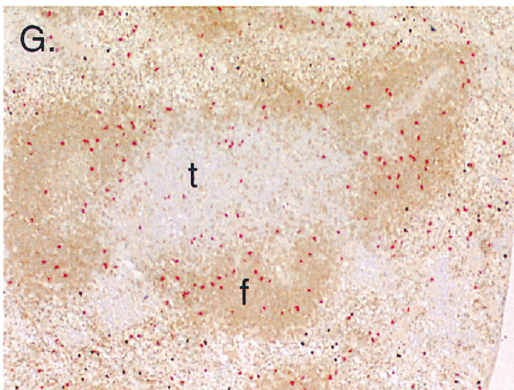
+/+ : +/+

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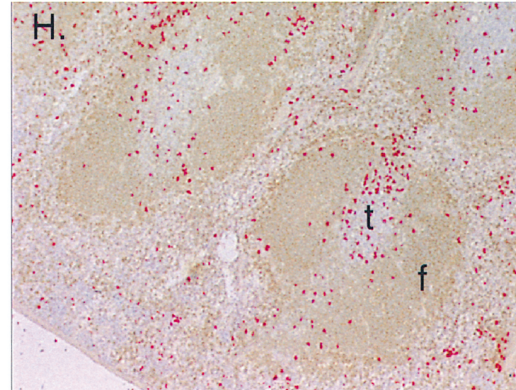me^V/me^V: +/+

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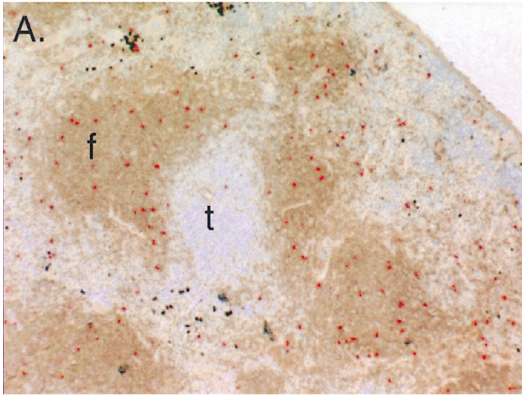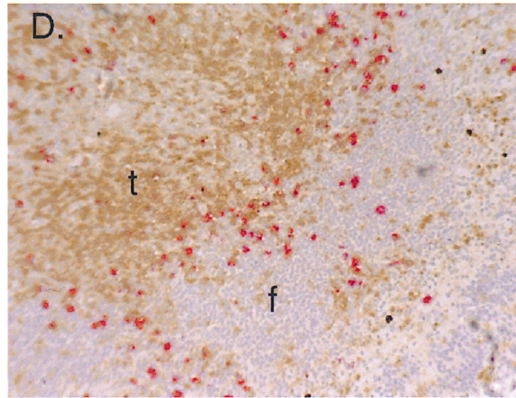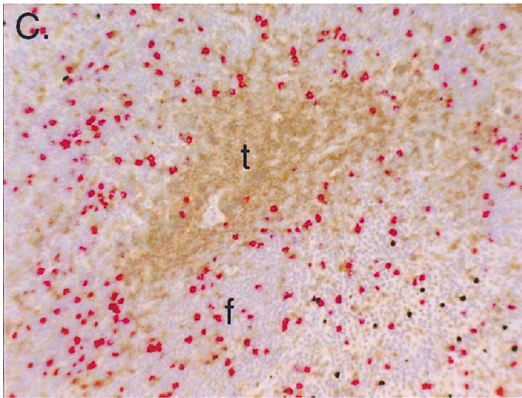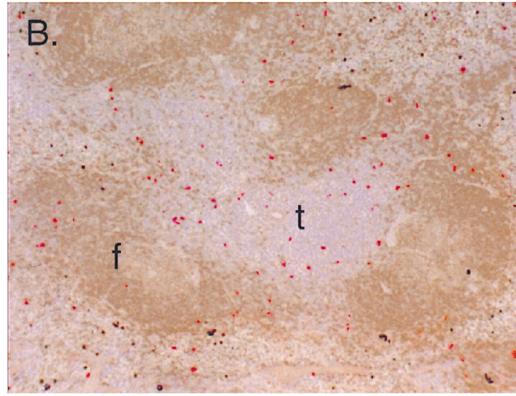
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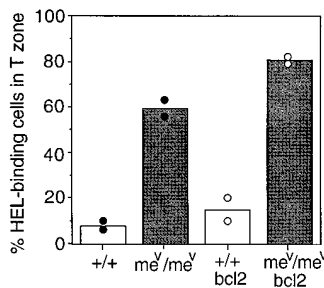me^V/me^V x bcl2 : +/+

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Figure 3. Percentage of Ig-transgenic B cells within the splenic white pulp that are located in the T cell zone. Cells were enumerated in sections from two mice double stained for T cell markers CD4 and CD8 and HEL-binding cells. All the HEL-binding cells present in the white pulp cord of each splenic cross-section were counted and cross-sections of white pulp cords were counted until a minimum of 100 HEL-binding cells had been counted for each spleen.

Follicular Exclusion of SHP1-deficient B Cells Is Not Rescued by Introduction of a bcl2 Transgene. To test whether the failure of me^v B cells to localize efficiently in lymphoid follicles was secondary to failure of the cells to survive, me^v mice were crossed with bcl2–transgenic mice (22), and me^v/me^v-Ig/bcl2 transgenic bone marrow was used for the construction of chimeras at a ratio of 20% me^v/me^v-Ig/bcl2–transgenic bone marrow and 80% wild-type nontransgenic bone marrow. Control chimeras were established using 2% wild-type Ig/bcl2-transgenic bone marrow and 98% nontransgenic bone marrow. Cells from the spleens of chimeric mice reconstituted for 5–6 wk were stained to determine the frequency of HEL-binding B cells. Constitutive expression of the bcl2 transgene in SHP1-deficient B cells led to an approximately fivefold increase in the number of these cells in the spleen (Fig. 1 *F*). When cell localization was examined, the exclusion of me^v B cells from follicles and accumulation in the T cell zone was even more marked than in the case of cells lacking the bcl2 transgene (Fig. 2 *H* and Fig. 3). The distribution of cells in control Ig/bcl2 chimeras did not differ notably from their distribution in the absence of the bcl2 transgene (Fig. 2 *G*). This strongly suggests that the failure of SHP1-deficient B cells to accumulate in lymphoid follicles is not secondary to cell death. Conversely, the higher frequency of SHP1-deficient Ig/bcl2-transgenic B cells in the T zone than in the case of cells lacking the bcl2 transgene suggests that many me^v/me^v B cells normally reach the T zone but undergo rapid cell death.

SHP1-deficient B Cells Localize in Lymphoid Follicles when Wild-type B Cells Are Lacking. Having observed antigen-independent exclusion of SHP1-deficient B cells from lymphoid follicles, we went on to ask whether the exclusion of these cells was dependent on competition between SHP1-deficient and wild-type B cells. This required assessing whether SHP1-deficient B cells localized in follicles or re-

mained distributed in the T zone when wild-type B cells were lacking. For this purpose, we established mixed bone marrow chimeras using 20% me^v/me^v Ig-transgenic bone marrow (or wild-type marrow in controls) and 80% Rag1$^{-/-}$ bone marrow. Since Rag1$^{-/-}$ bone marrow should contribute normally to the myeloid compartment, we expected that the effects of me^v/me^v-derived myeloid cells would continue to be diluted by wild-type (Rag1$^{-/-}$) myeloid cells. However, no mature B cells would be generated from Rag1$^{-/-}$ bone marrow, and hence, no wild-type B cells should be produced to compete with SHP1-deficient B cells in the periphery. Flow cytometric analysis of bone marrow from me^v/me^v–Ig:Rag1$^{-/-}$ chimeras established that immature SHP1-deficient B cells could develop in these animals (Fig. 1 *I*) and mature B cells with an IgD^{hi} conventional phenotype were detected in the periphery (Fig. 1 *J*). As in previous chimeras, many of the SHP1-deficient B cells in the periphery had downregulated IgM (Fig. 1 *J*). Strikingly, in the absence of competing normal B cells, the SHP1-deficient B cells in the spleens of these chimeric mice displayed a follicular distribution very similar to the distribution of wild-type cells in control chimeras (Fig. 4, *A* and *B*).

To further test whether SHP1-deficient B cells in me^v/me^v:Rag1$^{-/-}$ chimeras were located in follicles, we transferred fluorescently (CFSE) labeled wild-type B cells into several chimeric animals 1 d before tissue isolation. By transferring small numbers of wild-type B cells and providing only sufficient time for these cells to home normally to follicles, we minimized the possibility that the transferred cells would induce changes in the distribution of the endogenous SHP1-deficient B cells. After 16 h, the transferred wild-type B cells were present in the spleen in similar numbers to the endogenous SHP1-deficient B cells and were found intermingled with these cells (Fig. 4 *C*) confirming that the SHP1-deficient B cells were organized in follicles. The presence of MOMA-1–positive marginal zone macrophages at the border of the B cell clusters (Fig. 4 *C*, *blue staining*) also established their authenticity as follicles (28). To exclude the possibility that SHP1-deficient B cells no longer located in the T cell zone because of a paucity of wild-type T cells in the me^v/me^v–Rag1$^{-/-}$ chimeras, a further set of chimeras were constructed using a mixture of bone marrow from me^v/me^v-Ig–transgenic mice and mice with a disruption of the IgM (μ) heavy chain, which fail to produce B cells (24). Since T cell development is not disrupted in μ$^{-/-}$ animals, T cell production in these chimeras is expected to be identical to that in me^v/me^v:+/+ mixed bone marrow chimeras. Small numbers of CFSE-

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Figure 2. Exclusion of SHP1-deficient B cells from primary follicles and mantle zones of secondary follicles in mice containing wild-type B cells. Spleen sections from chimeric mice stained in red to detect Ig-transgenic cells (*A–E, G,* and *H*) and in brown to detect B220 (*A, B,* and *E–H*), or CD4 and CD8 (*C* and *D*). In *F*, a serial section to that in *E* is shown stained with peanut agglutinin (*red*) to detect germinal centers. *A* and *C* show distribution of +/+ Ig-transgenic B cells; *B, D,* and *E* show distribution of me^v/me^v Ig-transgenic B cells, *G* shows distribution of +/+ Ig/bcl2 double transgenic B cells, and *H* shows the distribution of me^v/me^v-Ig/bcl2 double transgenic B cells. Spleens are from nontransgenic mice reconstituted for 6 wk with mixtures of the following bone marrow: *A* and *C,* 5% +/+ Ig-transgenic and 95% nontransgenic; *B,* and *D–F,* 20% me^v/me^v-Ig–transgenic and 80% nontransgenic; *G,* 2% +/+ Ig/bcl2 double transgenic and 98% nontransgenic; *H,* 20% me^v/me^v-Ig/bcl2 double transgenic and 80% nontransgenic. *f,* follicle; *t,* T cell zone. Original magnification: *A, B,* and *E–H,* ×10 objective; *C* and *D,* ×20 objective. The figures are representative of tissue sections from the following number of chimeric mice: *A* and *C,* six mice; *B* and *D,* nine mice; *E* and *F,* three mice; *G,* two mice; and *H,* three mice.
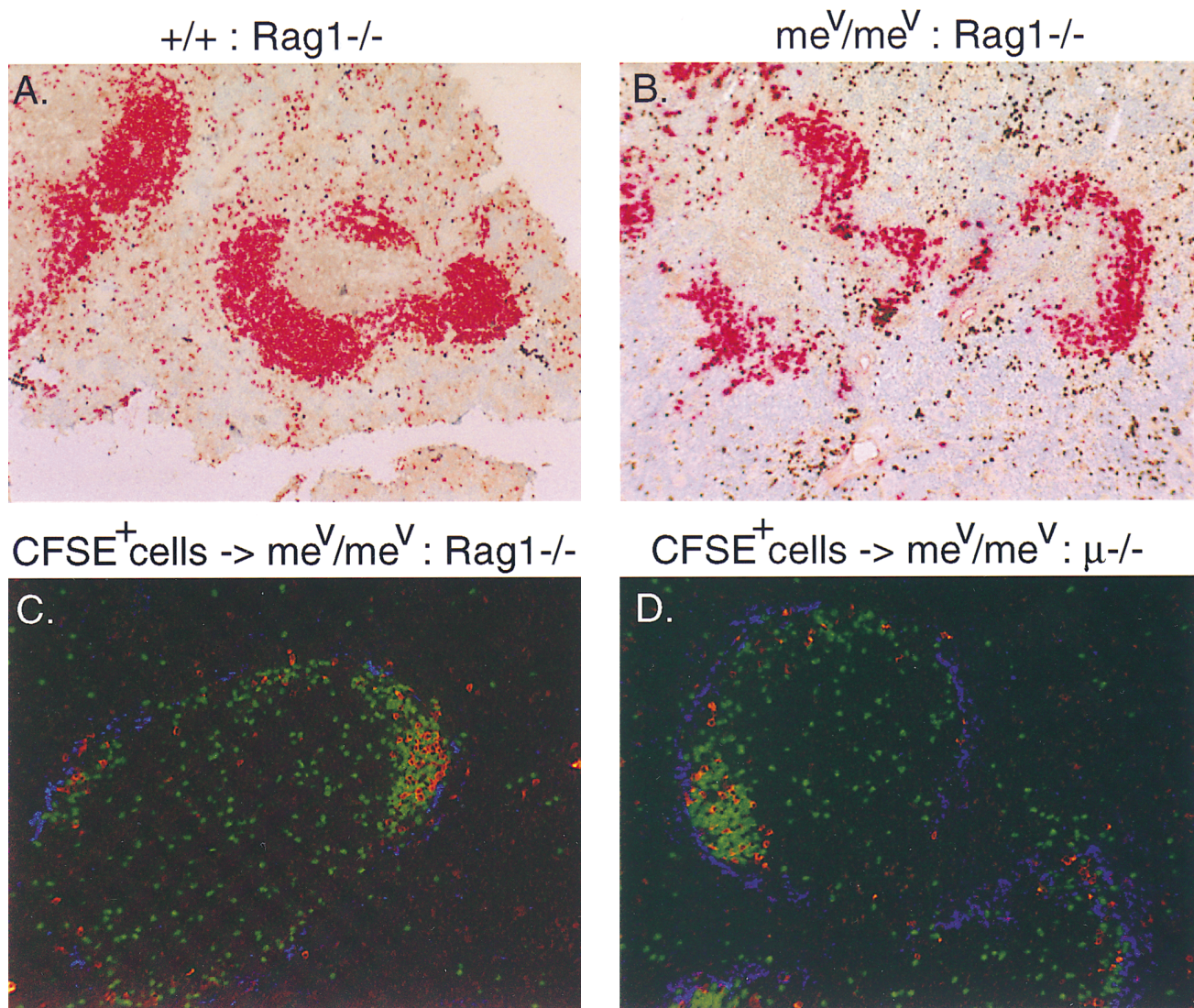
Figure 4. Follicular localization of *me^v* B cells in mice deficient in wild-type B cells. (*A* and *B*) Spleen sections from chimeric mice stained to detect Ig-transgenic cells (*red*) and CD4 and CD8 T cells (*brown*). (*C* and *D*) Spleen sections from chimeric mice that 16 h earlier received CFSE-labeled wild-type spleen cells. HEL-binding cells are stained red, MOMA-1–positive macrophages are stained blue, and transferred CFSE-labeled cells are detected as green cells. Mice had been reconstituted 6 wk earlier with: (*A*) 20% +/+ Ig-transgenic and 80% Rag1$^{-/-}$ bone marrow, (*B* and *C*) 20% *me^v/me^v* Ig-transgenic and 80% Rag1$^{-/-}$ bone marrow, and (*D*) 20% *me^v/me^v* Ig-transgenic and 80% μ$^{-/-}$ bone marrow. *A* is representative of two and *B* of seven mice. *C* and *D* are each representative of two mice. Original magnification: *A* and *B*, ×10 objective; *C* and *D*, ×20 objective.

labeled wild-type spleen cells were also transferred into these chimeras 16 h before analysis. Again, SHP1-deficient B cells were found to organize into follicles rather than locating within the T cell zone (Fig. 4 *D*).

SHP1-deficient B Cells Express BLR1. Studies in BLR1 knockout mice have established an important role for this orphan chemokine receptor in B cell homing to splenic follicles (8). To examine whether the exclusion of SHP1-deficient B cells from lymphoid follicles could reflect a failure to express BLR1, spleen cells from chimeric mice were stained with an affinity purified antiserum specific for the NH$_2$ terminus of BLR1. SHP1-deficient cells were found to express high levels of surface BLR1 (Fig. 5) and levels were similar in mice that contained competitor cells, where the

SHP1-deficient cells were excluded from follicles, and in animals that lacked competitor cells where the SHP1-deficient cells were follicular (data not shown). Immature (IgM$^+$IgD$^{lo/−}$) B cells by contrast expressed little or no BLR1 (Fig. 5).

Discussion

The findings above allow us to reach two conclusions: (*a*) SHP1 regulates intracellular signals controlling B cell positioning and (*b*) B cells can influence each others positioning through a process that cannot be explained by variation in antigen availability or presentation. These findings provide strong support for the model that at least two fac-
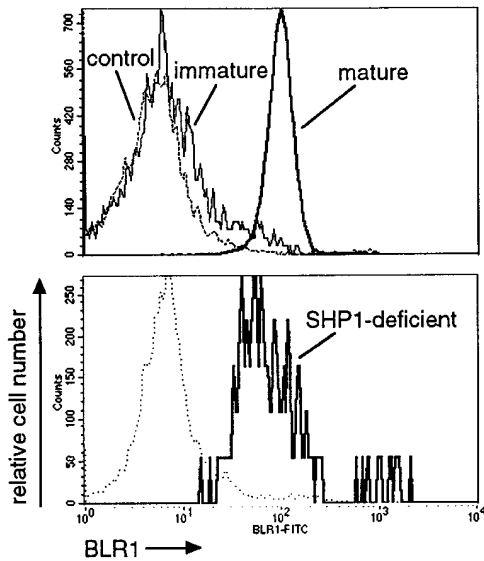
Figure 5. BLR1 expression on SHP1-deficient and wild-type B cells. (*Top*) BLR1 expression on IgM$^+$IgD$^{lo/-}$ bone marrow B cells (*immature*) and IgM$^+$IgD$^+$ spleen B cells (*mature*) from a +/+ Ig-transgenic mouse. (*Bottom*) BLR1 expression on B220$^+$ HEL-binding SHP1-deficient spleen cells from a mouse reconstituted 6 wk earlier with 20% *me*^{v}/*me*^{v} Ig-transgenic and 80% Rag1$^{-/-}$ bone marrow. The dotted line (*control*) shows staining with an irrelevant rabbit antiserum (*top*) or anti-BLR1 staining of T cells (*bottom*) as controls.

tors contribute to the follicular exclusion of mature B cells: intracellular signaling by the BCR and the presence or absence of competitor B cells without equivalent levels of intracellular signaling.

SHP-1 deficiency could cause B cells to be excluded from follicles in competitive circumstances by exaggerating signals from the BCR that oppose follicular tropism, or by interfering with delivery of maturation signals that promote follicular migration. Studies by MacLennan and coworkers have established that when immature B cells first enter the spleen, they are unable to migrate into follicles and instead locate in the outer T zone and in the red pulp (6, 32). What regulates the transition from an immature cell newly arrived in the periphery to a mature recirculating cell is poorly characterized and it is unclear whether the transition requires a positive signal, is limited by negative signals, or both. It seems likely that competency to enter follicles and become a recirculating cell relates at least in part to expression of BLR1, since this receptor is required for B cell entry into splenic follicles and it is not expressed on immature B cells (Fig. 4 and references 7, 26). Although it is conceivable that SHP1 deficiency interrupts B cell entry into follicles by inhibiting B cell maturation, we think this possibility is unlikely since the majority of SHP1-deficient B cells in the spleen express high levels of IgD and BLR1. Furthermore, immature *syk*$^{-/-}$ B cells do not enter follicles even when mature B cells are lacking (32), whereas the

SHP1-deficient cells localize within follicles in the absence of wild-type competitor B cells.

The distribution of SHP1-deficient B cells in the presence of competitor B cells is not identical to the distribution of antigen-engaged wild-type B cells (11). Although the latter cells tend to accumulate at the border of B and T cell zones, SHP1-deficient cells were more frequently dispersed through the T zone or located in marginal zone bridging channels between T zone and red pulp. Although some cells were also present in the red pulp, these are likely to be immature cells newly arriving in the spleen from the bone marrow. Overall, the distribution of cells in the white pulp cords is similar to antigen-binding B cells that also are receiving T cell help (11, 33) and suggests that in addition to downregulating signaling by the antigen receptor, SHP1 may also negatively regulate one or more pathways normally stimulated upon encounter with antigen-specific T cells. This is also suggested by the finding of anti-HEL antibody secreting plasma cells and HEL-binding germinal center cells in some of the chimeric animals. The failure of *me*^{v} B cells to accumulate in normal numbers as mature B cells in the periphery, even when containing a constitutively expressed bcl2 transgene or in mice lacking competitor cells, also suggests increased activation of intracellular pathways leading to cell death.

SHP1-mediated negative regulation of antigen-receptor signaling involves recruitment of the phosphatase to intracellular tyrosine-based inhibitory motifs (ITIMs) in the cytoplasmic domain of CD22 (34). The evidence that SHP1 plays a role in setting the basal level of antigen receptor signaling within B cells has recently been extended by the finding that mature B cells deficient in CD22, like SHP1-deficient B cells, have downmodulated surface IgM and increased class II and CD44 expression in the absence of added antigen (for review see reference 35). These findings support the notion that SHP1-deficient B cells have elevated basal signaling from the antigen receptor. The possibility that SHP1 regulates intracellular signaling pathways in B cells in addition to the antigen receptor has gained support from the finding of a family of molecules expressed in mature B cells that contain ITIMs. These molecules, which include the paired Ig-like receptors (PIRs; reference 36), leukocyte Ig-like receptor (LIR-1; reference 37), leukocyte-associated Ig-like receptor 1 (LAIR-1; reference 38), and Ig-like transcript 2 (ILT2; reference 39) are homologous to both the killer-inhibitory receptor (KIR) family of negative regulatory molecules expressed on natural killer cells and to CD22, and therefore are likely to act as negative regulators although the nature of their function is undefined. It will be important in future studies to determine the predominant molecules that recruit SHP1 in B cells and to characterize substrates of SHP1. Such studies should help define the signaling pathways that regulate expression of BLR1 and other molecules that function to control the localization and survival of mature B cells.

In summary, the findings reported here provide evidence that exclusion of mature B cells from follicles and accumu-

lation in the T cell zone is, in the absence of antigen, conditional on both elevated intracellular signaling and the presence of competitor B cells without such exaggerated signaling. Since interclonal competition among peripheral B cells may play a major role in determining the composition of the long-lived follicular B cell population (15), further efforts to understand the mechanism of competition are needed. In particular it will be important to define whether chemokine gradients are involved in positioning B cells in follicles or T zones and how these might be affected by follicular composition.

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