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Novel Roles for Lyn in B Cell Migration and Lipopolysaccharide Responsiveness Revealed Using Anti-Double-Stranded DNA Ig Transgenic Mice

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Lyn-deficient mice produce Abs against dsDNA, yet exhibit exaggerated tolerance to the model Ag hen-egg lysozyme. To investigate this apparent contradiction, and to further examine the function of Lyn in Ag-engaged cells, we have used an anti-dsDNA Ig transgenic model. Previously, looking at these anti-dsDNA B cells in Lyn-sufficient BALB/c mice, we showed that they are regulated by functional inactivation (anergy). In the absence of Lyn, these anti-dsDNA B cells remain unable to secrete Ab. This suggests that functional inactivation of anti-dsDNA B cells does not depend on Lyn, and that the anti-dsDNA Abs that are produced in lyn−/− mice arise from a defect in another mechanism of B cell tolerance. Although the anti-dsDNA B cells remain anergic, Lyn deficiency does restore their ability to proliferate to LPS. This reveals a novel role for Lyn in mediating the LPS unresponsiveness that normally follows surface Ig engagement. Furthermore, Lyn deficiency leads to an altered splenic localization and EBV-induced molecule 1 ligand chemokine responsiveness of anti-dsDNA B cells, as well as an absence of marginal zone B cells, suggesting additional roles for Lyn in controlling the migration and development of specific B cell populations. The Journal of Immunology, 2001, 166: 3710–3723.

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2 Address correspondence and reprint requests to Dr. Jan Erikson, The Wistar Institute, Room 276, 3601 Spruce Street, Philadelphia, PA 19104. E-mail address: jan@wistar.upenn.edu

3 Abbreviations used in this paper: BCR, B cell Ag receptor; AP, alkaline phosphatase; BLC, B lymphocyte chemotactant; BM, bone marrow; ELC, EBV-induced molecule 1 ligand chemokine; GC, germinal center; HEL, hen-egg lysozyme; MZ, marginal zone; PALS, periarteriolar lymphoid sheath; Tg, transgene.

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we used the VH3H9 Ig transgene (Tg) to follow a population of BM-generated anti-dsDNA B cells. Previously, we have described a regulated phenotype for these anti-dsDNA B cells in BALB/c mice: in vitro, they do not proliferate in response to LPS stimulation; in vivo, they are developmentally arrested, halted at the 7/B interface in the splenic white pulp, and fail to secrete Ab (19, 22). Strikingly, in Fas-deficient mice, a murine model of systemic lupus erythematosus, anti-dsDNA B cell anergy is not maintained; the anti-dsDNA B cells exhibit phenotypic differences and spontaneously secrete autoantibody (23). In lymphocytes, we find that anti-dsDNA B cells exhibit the same anergic cell surface phenotype seen in the presence of Lyn. Additionally, they are unable to secrete Ab, indicating that Lyn is not required for the induction or maintenance of B cell anergy. However, this study does reveal that Lyn significantly impacts several other B cell responses to autoantigen encounter. In particular, we documented previously undescribed roles for Lyn in controlling the LPS responsiveness and migration of Ag-engaged B cells.

Materials and Methods

Mice

Lyn−/− mice (backcross 4 onto the C57BL/6 background from a mixed 129/C57BL/6 background) were obtained from E. Pure (The Wistar Institute, Philadelphia, PA), who received them originally from C. Lowell (University of California, San Francisco, CA). VH3H9 BALB/c mice were crossed with lymphocytes, and the resulting progeny were intercrossed to create Tg lymphocytes, and VH3H9 lymphocytes, and VH3H9 lymphocytes. Some of these Tg lymphocytes and VH3H9 lymphocytes were also mated to generate experimental mice; similar results were obtained from both generations of lymphocytes. In this work, the designation Lyn−/− refers to Lyn-sufficient mice of this genetic background, distinguishing them from BALB/c mice, which are also Lyn sufficient. The mice were bred and maintained in a specific pathogen-free room at The Wistar Institute animal facility, and they were sacrificed for experiments at 6–10 wk of age. All animal work was conducted in accordance with institutional guidelines.

PCR amplification of tail DNA was used to determine genotypes of mice. VH3H9 PCR was conducted as described (24). The presence of a wild-type lyn locus was determined as described (10). The presence of a neomycin insert (indicating a mutant lyn locus) was detected by PCR using primers derived from sequences in the 5′ and 3′ portions of the neomycin insert. The sequences used were: 5′-TACGGCAGGGGCGCGGTCTTT-3′ and 5′-ATCAG CGACGGTGTCATGCAG-3′. PCR conditions were 94°C for 5 min; 70°C for 1 min, 72°C for 1 min, and 94°C for 1 min for 40 cycles; 70°C for 1 min; and 72°C for 5 min.

Cell preparations

One femur and one tibia were removed from each mouse for BM analysis, with the cells from both bones being pooled. Single cell suspensions from spleen and BM were prepared by disrupting the organ of interest and passing the resulting solution through a 70-µm cell strainer (Fisher Scientific, Pittsburgh, PA). RBC were then removed with RBC lysing buffer (Sigma, CA). Spleen cells were stained with anti-B220 biotin/streptavidin Red670 and anti-CD19 PE. Gates were drawn as shown in Fig. 1, with the B220 IgM gate being used to calculate the number of IgM cells/spleen. The percentage of B220+ IgM+ cells of live cells (based on forward and side scatter) was multiplied by the total number of live cells per spleen (quantified by trypan blue exclusion using a hemacytometer and light microscope). The number of IgM cells/spleen was calculated in a similar fashion. Because staining with anti-IgM FITC shows that the B220+ IgM+ population consists of IgM cells (data not shown), the B220+ IgM+ gate was used to calculate the number of IgM cells/spleen.

In vitro proliferation assay

Spleen cells were labeled with CFSE (Molecular Probes, Eugene, OR), as described (26). CFSE-labeled spleen cells (2 × 10^6/ml) were then cultured in either medium alone (RPMI 1640/10% FCS/5 × 10^−3 M 2-ME) or LPS (10 µg/ml, Sigma). Cells were removed from culture after 4 days to determine viability, as assessed by trypan blue exclusion under a light microscope, and proliferation. Proliferation was measured by flow cytometry as a decrease in CFSE intensity relative to unstimulated cells. Each cell division is accomplished by a decrease in CFSE intensity by one-half. Percent of recovery was determined by dividing the number of cells recovered on day 4 by the number of cells initially placed in culture.

B cell hybridoma production

Spleen cells from an 8-wk-old VH3H9 lymphocyte mouse were cultured for 2 days with 10 µg/ml LPS (Sigma). Subsequently, hybridomas were generated, and Ab isotype and specificity were determined as described (27).

Immunohistochemistry

Spleens were suspended in OCT (Fisher Scientific), frozen in 2-methyl-butane cooled with liquid nitrogen, sectioned, and fixed with acetone. The 5- to 8-µm spleen sections were stored at −20°C and then stained according to the protocol described (28). Briefly, the sections were blocked using PBS/5% normal goat serum (Sigma)/0.1% Tween 20, and then stained with RA3-6B2 biotin (anti-B220; PharMingen), GK1.5 biotin (anti-CD4; grown as supernatant and conjugated), MOMA-1 (antimarginal zone (MZ) metallophilic macrophages; Bachem, Torrance, CA), Cy34.1 FITC (anti-CD22; PharMingen), or anti-IgA alkaline phosphatase (AP; Southern Biotechnology Associates, Birmingham, AL). Streptavidin HRP (Southern Biotechnology Associates), polyclonal anti-rat HRP (Jackson ImmunoResearch, West Grove, PA), and anti-FITC AP (Sigma) were used as secondary Abs. HRP and AP were developed using the substrates 3-amin-9-ethylcarbazole and Fast Blue BB base (Sigma), respectively.

Chemotaxis assays

Chemotaxis assays were performed in 5-µm Transwell plates (Corning Costar, Cambridge, MA). Single cell suspensions were prepared in endotoxin-free RPMI 1640 (Life Technologies) containing 10% FCS and 5 × 10^−3 M 2-ME. EBV-induced molecule 1 ligand chemokine (ELC) and B lymphocyte chemotactic factor (BLC) (R&D Systems, Minneapolis, MN) were diluted in the same medium. Transwells were preincubated with medium for 10 min at 37°C. Subsequently, this medium was removed, and chemokine solution (600 µl) was added to the bottom chamber of the Transwell. A total of 1 × 10^5 spleen cells (in 100 µl) was added to the upper well, and cells were allowed to migrate for 4 h at 37°C. Migrated cells were harvested, quantified by trypan blue exclusion, and identified by flow cytometry.

Cricthidia luciliae assay

The presence of anti-dsDNA Abs in serum was detected using fixed, peroxidase-conjugated C. luciliae (29) as the substrate (Antibodies, Davis, CA). Serum samples were tested at serial 10-fold dilutions from 1/100 to 1/1,000,000. Anti-dsDNA Ab binding (defined as staining of the kinetoplast) was detected either with a combination of FITC-conjugated goat anti-mouse IgG and anti-mouse IgM reagents, or with a FITC-conjugated goat anti-mouse IgA reagent (Southern Biotechnology Associates). The samples were then visualized under a fluorescent microscope and scored without knowledge of age or genotype of the mice. The serum titer was defined as the reciprocal of the last dilution at which kinetoplast staining was seen.

Statistical analysis

Statistical significance was determined using an unpaired, two-sample Student’s t test and Instat software.

Results and Discussion

The anti-dsDNA Ig Tg model

The VH3H9 Tg is an Ig heavy chain Tg that, in combination with the endogenous A1-light chain, forms an anti-dsDNA Ab (27, 30).
By using anti-\( \lambda \) reagents, we can specifically identify anti-dsDNA B cells within VH3H9 Tg mice. Endogenous k-light chains can also combine with the VH3H9 Tg, yielding both anti-DNA Abs and nonautoimmune specificities (24, 27, 30); therefore, the Ig\( \lambda \) anti-dsDNA B cells can be studied within a diverse B cell repertoire. Using this approach, we have examined anti-dsDNA B cells in a variety of contexts, including in this study in lyn\(^{-/-}\) mice.

**Anergic anti-dsDNA B cells are not deleted in lyn\(^{-/-}\) mice**

We have previously reported that Ig\( \lambda \) anti-dsDNA B cells from VH3H9 BALB/c mice are present in both the BM and spleen and, consistent with Ag encounter, express a decreased level of surface Ig compared with Tg\(^{-/-}\) Ig\( \lambda \) B cells (19). This remains true for the Ig\( \lambda \) anti-dsDNA B cells in the lyn\(^{+/+}\) genetic background investigated in this study (Fig. 1A). Ig\( \lambda \) cells are also present in the BM and spleens of VH3H9 lyn\(^{-/-}\) mice with decreased levels of surface Ig, suggesting that whether or not Lyn is present, the anti-dsDNA B cells see their Ag at the same early stage of development. Additionally, their absolute number is the same in VH3H9 lyn\(^{-/-}\) spleens as in VH3H9 lyn\(^{+/+}\) (Fig. 1B).

The ability of Ig\( \lambda \) anti-dsDNA B cells to persist in VH3H9 lyn\(^{-/-}\) mice is particularly striking when compared with the dramatic reduction in the number of VH3H9/Ig\( k \) cells. Consistent with published data, we found that the number of B cells in Tg\(^{-/-}\) lyn\(^{-/-}\) mice was decreased 54–89% (10, 11, 14), and both Ig\( \lambda \) and Ig\( k \) cell numbers were equally reduced. Although similar reductions in overall B cell number were found in VH3H9 lyn\(^{-/-}\) mice, in this case the decrease occurred only in the Ig\( k \) population. Thus, compared with the majority of B cells in VH3H9 lyn\(^{-/-}\) mice, Ig\( \lambda \) anti-dsDNA B cells appear better able to survive in the absence of Lyn. One explanation for this is that anti-dsDNA B cells are rescued by encounter with Ag. In this regard, anti-Ig treatment of lyn\(^{-/-}\) B cells was shown to result in hyperactivation of Akt, a B cell survival factor (31, 32).

Interestingly, lyn\(^{-/-}\) anti-dsDNA B cells also have a survival advantage over lyn\(^{-/-}\) anti-HEL B cells that have encountered Ag (20). One obvious difference between lyn\(^{-/-}\) anti-HEL B cells (which are deleted) and lyn\(^{-/-}\) anti-dsDNA B cells (which survive) is the Ag specificity. This suggests that the survival of lyn\(^{-/-}\) B cells (and possibly the extent of Akt activation) is not only

**FIGURE 1.** Ig\( \lambda \) anti-dsDNA B cells are present with decreased surface Ig. A. BM (left) and spleen (right) cells from Tg\(^{-/-}\) (top) and VH3H9 (bottom) mice were stained with Abs against B220 and Ig\( \lambda \). The mean fluorescence intensity is given for the B220<sup>-</sup> Ig\( \lambda ^{+} \) cells in the boxed region to the right. B. Using the gates shown in A, the total number of splenic Ig\( \lambda ^{+} \) (left) and Ig\( k ^{+} \) (right) cells was calculated as described in Materials and Methods. Brackets indicate significant differences with \( p \) values as indicated. There is no significant difference between the number of Ig\( \lambda \) anti-dsDNA B cells in VH3H9 lyn\(^{+/+}\) and VH3H9 lyn\(^{-/-}\) mice (\( p = 0.1855 \)). These are representative plots of \( n \geq 6 \) mice of each genotype.
linked to Ag encounter, but is also determined by the strength of signaling through surface Ig. Given that the nature of the in vivo Ag regulating anti-dsDNA B cells has not been defined, this is difficult to directly evaluate.

**Maintenance of the anergic phenotype in lyn−/− anti-dsDNA B cells**

Previously, using flow cytometric analysis, we established that anti-dsDNA B cells in VH3H9 BALB/c mice display an altered cell surface phenotype that correlates with their functional inactivation (19). This phenotype, which includes decreased levels of B220, CD21/35, CD22, and CD23, is also found in the VH3H9 lyn−/− mice investigated in this work (Fig. 2). Before examining cell surface marker expression in VH3H9 lyn−/− mice, we established the phenotype of B cells from Tg ( lyn−/−) mice. The maturation status of splenic lyn−/− B cells has been controversial, with some groups (11, 20) suggesting no defects in B cell development and one group reporting a block in progression from the B220low HSA− (immature) to B220high HSA− (mature) stage (13). In our study, Tg(−) lyn−/− B cells are able to mature and express wild-type levels of B220 and CD22, with the ratio of immature to mature cells being no different from that seen in lyn−/+ mice (Fig. 2).

Igκ anti-dsDNA B cells in VH3H9 lyn−/− mice exhibit the same cell surface profile seen in VH3H9 lyn−/+ mice, with reduced levels of B220, CD21/35, CD22, and CD23 (Fig. 2). Thus, by flow cytometry, the lyn−/− Igκ anti-dsDNA B cells continue to have a phenotype that correlates with anergy. Of note, in the absence of Lyn, the majority of VH3H9/Igκ cells also express little to no CD21/35 and CD23. These are representative plots of n = 6 mice of each genotype.

To verify the functional inactivation of Igκ anti-dsDNA B cells, serum samples from 40-wk-old Tg(−) and VH3H9 lyn−/− mice were tested for anti-dsDNA Abs by the C. luciliae assay. Confirming previous reports (10, 11), high titers of autoantibodies were detected in Tg(−) lyn−/− mice (mean titer = 1.50 × 10³; n = 17). This contrasts with Lyn-sufficient mice, which have undetectable levels of anti-dsDNA Abs (titers <10; n = 10). Both Igκ and Igλ Abs contributed to serum titers in 15 of 17 Tg(−) lyn−/− mice; in the remaining two mice, only Igκ anti-dsDNA Abs were detected. We have previously reported that for the MRL-lpr/lpr mouse model of lupus, autotbody production is not affected by the presence of the VH3H9 Tg, and Igλ autoantibodies arise at the same time as Igκ autoantibodies (23). However, in VH3H9 lyn−/− mice, anti-dsDNA Ab titers are reduced (mean titer = 1 × 10³; n = 13), and, importantly, Igκ anti-dsDNA Abs are not detected.

Given that anti-dsDNA B cell anergy remains intact in the absence of Lyn, the mechanism for anti-dsDNA Ab production in lyn−/− mice remains unknown. In terms of BM-induced tolerance, anti-dsDNA B cells are a heterogeneous group that are regulated not only by anergy, but also by deletion. For example, anti-dsDNA B cells that use the VH3H9 Tg in combination with the Vκ4-light chain are deleted in the BM (33). One possibility, then, is that deletion of anti-dsDNA B cells in the BM is defective. This could be tested by tracking the fate of VH3H9/Vκ4 anti-dsDNA B cells in the absence of Lyn.

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**FIGURE 2.** Cell surface phenotype of splenic lyn−/− B cells. Histograms (gated as in Fig. 1) show representative staining of Igκ cells (bold histogram) overlaying staining of Igλ cells (thin histogram). Bold histograms have been scaled 10X to allow for comparison of the smaller pool of Igκ cells with Igλ cells. Except for lacking a CD21/35high peak, Tg(−) lyn−/− B cells closely resemble Tg(−) lyn+/− B cells. Regardless of Lyn expression, Igκ anti-dsDNA B cells in VH3H9 mice look strikingly different from Tg(−) cells, expressing lower levels of CD21/35, CD22, and CD23, as well as slightly lower levels of B220 (15–35% lower, p = 0.0371). In VH3H9 lyn−/− mice, the majority of Igκ cells also express low levels of CD21/35 and CD23. These are representative plots of n = 6 mice of each genotype.
A second possibility is that although BM-induced tolerance remains intact, there is a failure to regulate anti-dsDNA B cells produced in GCs. We have previously suggested that this is the case for bcl-2 Tg mice (34). Strikingly, in both lyn−/− and bcl-2 Tg mice, autoantibody production is reduced by the presence of the VH3H9 Tg. One explanation for this is that the VH3H9 Ig Tg increases production of autoreactive cells in the BM, where they are appropriately regulated, and by doing so limits the pool of nontolerized B cells available for recruitment into a GC. This would predict that the B cell repertoire in VH3H9 lyn−/− mice contains a much larger fraction of anergic B cells than Tg lyn−/− mice. Consistent with this, a large proportion of the Igκ population in VH3H9, but not Tg lyn−/−, lyn−/− mice appears altered, expressing low levels of CD21/35 and CD23 (Fig. 2).

Anti-dsDNA B cells are LPS responsive in the absence of Lyn
In addition to the induction of tolerance, there are many other ways in which B cells can be altered by Ag encounter. For example, B cells that have received an initial signal through the BCR appear hyporesponsive to B cell mitogens such as LPS (35). To see whether Lyn is involved in this process, we tested the response of lyn−/− anti-dsDNA B cells to LPS stimulation. We have previously noted that Igκ anti-dsDNA B cells in several genetic backgrounds (BALB/c, MRL+/−, and MRL-lpr/lpr) proliferate in response to a combination of CD40L + IL-4, but are markedly deficient in their response to LPS. Not only do the anti-dsDNA B cells fail to proliferate, but they also do not survive (22). The ability of lyn−/− B cells to proliferate in response to LPS has been disputed, with two groups reporting impaired proliferation in the absence of Lyn (11, 14) and one group finding no change (10). In this study, looking at Tg(lyn−/−) mice, we show that although lyn−/− B cells proliferate to LPS, they do not divide as many times as lyn+/+ B cells, as measured by decreases in CFSE intensity (Fig. 3, left panels). This could be explained by recent studies demonstrating that Lyn is essential to signaling from the Toll-like receptor protein RP105 (36), which in turn is necessary for optimal B cell responses to LPS (37).

Given that signals through Ig are reported to impair proliferation to LPS (35), as does the absence of Lyn, lyn−/− anti-dsDNA B cells might be expected to exhibit profound deficits in their response to LPS. However, remarkably, Lyn deficiency actually restores their ability to proliferate in the presence of LPS (Fig. 3, right panels). This suggests that Lyn is crucial to the pathways by which BCR signaling inhibits the LPS response. In support of this idea, it has been shown that coligation of the FcγRIIb (from which Lyn mediates signaling) enhances the inhibitory effect of surface Ig engagement on RP105-induced proliferation (38). Thus, Lyn

**FIGURE 3.** Proliferative response to LPS. Spleen cells were labeled with CFSE and cultured for 4 days in LPS. Cells were then harvested and stained with Abs against B220 and Igλ. Histograms show CFSE staining of Igλ (bold histograms) and Igκ (thin histograms) B cells overlaying the nonproliferating T cells (dotted histograms). Because Igκ B cells greatly outnumber Igλ B cells, the bold histograms were scaled up to allow for comparison of the two populations. Few Igλ anti-dsDNA B cells from VH3H9 lyn−/− mice remain after 4 days in culture (7.88 ± 5.06% recovery), and many have proliferated (n = 4 for VH3H9 lyn−/−; n = 3 for all other genotypes).

**FIGURE 4.** Alterations in lyn−/− spleens. A, Serial spleen sections from the indicated mice were stained with Abs against Igλ (blue) and either CD22 (brown; top) or CD4 (brown; middle). In Tg lyn−/− mice, Igλ cells localize with the other B cells, whereas in VH3H9 lyn−/− mice, the Igλ anti-dsDNA B cells accumulate at the T/B interface. Strikingly, in VH3H9 lyn−/− mice, the Igλ anti-dsDNA B cells are found spread throughout the PALS. B, Spleen sections were stained with Abs against CD22 (blue) and MOMA-1 (brown). MOMA-1 identifies metallophilic MZ macrophages and can be used to indicate the boundary between follicular and MZ B cells (41). Lyn−/− mice exhibit a decrease in the MZ B cell population located beyond MOMA-1 (n = 6 mice of each genotype).
transduces signals from at least one receptor that can affect Ig-mediated inhibition of the LPS response.

The altered LPS responsiveness of lyn\(^{-/-}\) anti-dsDNA B cells was also reflected in hybridomas generated from VH3H9 lyn\(^{-/-}\) B cells. In previous experiments, anti-dsDNA B cells were not captured in hybridoma panels generated after LPS stimulation (24). However, after LPS stimulation of VH3H9 lyn\(^{-/-}\) B cells, 6 of 102 Ig\(^{+}\) hybridomas secreted Ig\(\lambda\) autoantibody (data not shown).

**Altered localization and ELC responsiveness of anti-dsDNA B cells**

We have previously reported that anti-dsDNA B cells in VH3H9 BALB/c mice are restricted from entering the B cell follicles of the splenic white pulp, localizing instead at the interface between the periarteriolar lymphoid sheath (PALS) and the B cell follicle (19). Anti-dsDNA B cells also line up at the T/B interface in VH3H9 lyn\(^{-/-}\) mice (Fig. 4A). To determine whether Lyn affects the migration of Ag-experienced B cells, spleen sections from lyn\(^{-/-}\) mice were stained with anti-CD22 and anti-Ig\(\lambda\), or anti-CD4 and Ig\(\alpha\). In Tg\(^{-/-}\) lyn\(^{-/-}\) and lyn\(^{+/+}\) mice, Ig\(\alpha\) cells disperse throughout the B cell follicles. In VH3H9 lyn\(^{-/-}\) mice, as in VH3H9 lyn\(^{+/+}\) mice, Ig\(\lambda\) anti-dsDNA B cells are excluded from the B cell follicles; strikingly, however, instead of lining up at the T/B interface, the Ig\(\lambda\) cells are spread throughout the PALS.

One mechanism to account for the altered localization of lyn\(^{-/-}\) anti-dsDNA B cells is a change in their chemokine responsiveness. To examine this possibility, we studied migration in response to BLC, which promotes trafficking to the B cell follicle (39), and ELC, which can mediate homing to the T cell area (40). No consistent differences in baseline migration (migration at 0 ng/ml of chemokine) were seen between lyn\(^{+/+}\) and lyn\(^{-/-}\) B cells (Fig. 5). Additionally, no consistent differences in BLC responsiveness were seen between lyn\(^{+/+}\) and lyn\(^{-/-}\) B cells with or without the VH3H9 Tg (data not shown). In response to ELC, B cells and T cells from Tg\(^{-/-}\) lyn\(^{+/+}\) and Tg\(^{-/-}\) lyn\(^{-/-}\) mice migrated to a similar extent (Fig. 5A and data not shown). However, anti-dsDNA B cells from VH3H9 lyn\(^{-/-}\) mice exhibited a significantly greater chemotactic response than did those from VH3H9 lyn\(^{+/+}\) mice (Fig. 5B). Thus, Lyn does not seem to affect ELC responsiveness in resting B cells; however, it does appear to play a regulatory role after BCR engagement. This increased ELC responsiveness could explain why lyn\(^{-/-}\) anti-dsDNA B cells localize to the T cell area, where ELC is produced.

**Absence of MZ B cells in lyn\(^{-/-}\) mice**

A second alteration in splenic architecture was also noted in lyn\(^{-/-}\) mice. Staining with MOMA-1 (to mark MZ macrophages (41)) and anti-CD22 shows that B cells fail to fully populate the MZ in lyn\(^{-/-}\) mice (Fig. 4B). Compared with lyn\(^{+/+}\) mice, there is a clear decrease in the B cell population that usually extends beyond the ring of MOMA-1. Additionally, by flow cytometry, it is evident that lyn\(^{-/-}\) mice lack the CD21/35\(^{high}\) population of B cells (21) (Fig. 4). This decrease in the lyn\(^{-/-}\) B cell population was also reflected in hybridomas generated from VH3H9 lyn\(^{-/-}\) Tg (data not shown), indicating that Lyn deficiency does not affect the migration of lyn\(^{-/-}\) B cells, it increases the ELC responsiveness of anti-dsDNA B cells. Shown are the combined results from six experiments, with n \(\geq\) 4 mice of each genotype.

**FIGURE 5.** Chemotactic activity of ELC on Ig\(\lambda\) B cells. A total of 10\(^6\) spleen cells from Tg\(^{-/-}\) (A) and VH3H9 (B) mice was placed in the upper chamber of a Transwell filter. The lower chambers contained concentrations of ELC as indicated. Graphs show the percentage of input lyn\(^{-/-}\) (○) and lyn\(^{+/+}\) (□) Ig\(\lambda\) B cells that migrated to the lower chamber. Although Lyn deficiency does not affect the migration of lyn\(^{-/-}\) B cells, it increases the ELC responsiveness of anti-dsDNA B cells. Shown are the combined results from six experiments, with n \(\geq\) 4 mice of each genotype.
several novel aspects of Lyn function after Ig engagement in vivo. Lyn appears to regulate B cell migration after Ag encounter; in the absence of Lyn, anti-dsDNA B cells exhibit alterations in splenic localization and responsiveness to ELC. Additionally, the absence of Lyn restores the ability of anti-dsDNA B cells to respond to LPS, implicating Lyn in the suppression of LPS responses after Ig engagement. Finally, MZ B cells are absent in lyn−/− mice, demonstrating that Lyn is crucial to the development and/or localization of this B cell population.

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