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EBI2 Operates Independently of but in Cooperation with CXCR5 and CCR7 To Direct B Cell Migration and Organization in Follicles and the Germinal Center

Dominique Gatto,*† Katherine Wood,* and Robert Brink*†

Migration of B cells within lymphoid follicles is controlled by the chemokine receptors CXCR5 and CCR7 and the G-protein-coupled receptor EBI2 (GPR183). Whereas CXCR5 and CCR7 are known to mediate migration toward their respective chemokine ligands, it is unclear whether EBI2 acts by modulating these processes or by directly mediating chemotaxis toward its own spatially restricted ligand. It is also unknown how signals from these three receptors are integrated to control B cell localization. To answer these questions, we generated compound knockout mice deficient in expression of EBI2, CXCR5, or CCR7. Analysis of these mice revealed that EBI2 mediates B cell migration toward the outer areas of follicles and to bridging channels of the spleen independent of both CXCR5 and CCR7. Migratory signals delivered by EBI2 were shown to control B cell organization within the spleen and to be particularly important for positioning activated B cells in the early stages of Ab responses. An additional minor role for EBI2 was identified in the organization and affinity maturation of B cells in germinal centers. Thus, EBI2-mediated chemotaxis provides a third dimension to B cell migration that balances and integrates with the inputs from CXCR5 and CCR7 to determine B cell positioning.

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The organized architecture of secondary lymphoid tissues segregates lymphocytes into distinct compartments, which constitute B cell follicles, T cell areas, germinal centers (GCs) and the splenic marginal zone. The basis for the organization of lymphocytes is provided by the spatial and temporal control of the expression of chemoattractants and their receptors, which also provide the migratory signals that guide B and T cell populations during humoral and cellular immune responses. Homing of naive B cells to follicles is mediated by the chemokine receptor CXCR5, which is expressed at high levels on B cells. Thus, naive B cells predominantly respond to the CXCR5 ligand CXCL13, which is produced by stromal cells in B cell follicles (1, 2). Ag-induced activation of B cells results in rapid upregulation of the chemokine receptor CCR7, which directs their movement to the T zone–B zone boundary, toward the two CCR7 ligands CCL19 and CCL21 produced in T cell areas (3, 4). As the B cell response progresses, regulated expression of CXCR4 mediates the segregation of GC B cells into a dark zone (DZ) and a light zone (LZ) and directs the migration of plasma cells to the splenic red pulp and bone marrow (5, 6).

Recent studies have identified the G protein-coupled receptor EBI2 (also known as GPR183) as an additional receptor regulating the positioning of naive and activated B cells in follicles (7, 8). EBI2 has been reported to be most homologous to members of the lipid and purine GPCR family (9–11). Although EBI2 was until recently an orphan GPCR, its ligand was recently identified to be the oxysterol 7α,25-dihydroxycholesterol (12, 13). EBI2 is expressed at high levels on naive B cells, and this high constitutive expression of EBI2 is increased further after activation (7, 8, 14, 15). In contrast, GC B cell differentiation is associated with a strong downregulation of EBI2 expression (7, 8, 14, 15), and differential expression of EBI2 in GC DZ and LZ populations has been reported recently (16). EBI2-deficient mice display normal architecture of secondary lymphoid organs, but EBI2-deficient B cells were found to have an abnormal migration pattern and severe defects in the generation of Ab responses (7, 8). Specifically, expression of EBI2 was essential for the movement of activated B cells to extrafollicular sites and the induction of early plasmablast responses (7). Conversely, downregulation of EBI2 enabled B cells to access the center of follicles and was required for efficient GC formation (7, 8). Thus, EBI2 collaborates with the chemokine receptors CXCR5, CCR7, and CXCR4 to position B cells appropriately for mounting Ab responses.

Despite the integral role of EBI2 in B cell migration, the mechanism by which it functions is still unclear. In particular, it is unknown whether EBI2 mediates chemotaxis directly or indirectly by modulating the chemotactic signals delivered through CXCR5 or CCR7, or both. Molecular analysis of EBI2 has suggested that this receptor may have constitutive activity (9, 17), but the study of EBI2-deficient B cells has provided evidence that EBI2 regulates B cell localization through its recently identified ligand. Moreover, as EBI2, CXCR5, and CCR7 are coexpressed on B cells, it is unclear to what extent they each control the position and organization of naive and activated B cells. To address these questions, we generated different combinations of compound knockout mice, deficient in expression of EBI2, CXCR5, and CCR7 and analyzed the migratory pattern and immune response of B cells from these mice. These studies reveal that EBI2 directly mediates CXCR5-
and CCR7-independent chemotaxis toward the outer regions of the splenic white pulp, presumably where its ligand is concentrated. We show that EBI2-mediated chemotaxis acts in concert with the migratory signals delivered by CXCR5 and CCR7 to localize B cells at various positions within lymphoid follicles depending on the balanced integration of these three independent chemotactic stimuli. The regulation of B cell migration by EBI2 is shown to have an important role in controlling the positioning of activated B cells within follicles in the early phases of B cell responses and to have a minor function in DZ and LZ B cell differentiation and affinity maturation during the GC reaction.

Materials and Methods

**Mice**

EBI2−/− and SWHEL mice have been described previously (7, 16). Cxcr5−/− and Ccr7−/− mice were purchased from The Jackson Laboratory; CD45.1 congenic C57BL/6 (B6.STJL-prkdcScid) mice were obtained from the Animal Resources Centre (Perth, Australia). All mice were maintained on a C57BL/6 genetic background and bred and housed in a specific pathogen-free environment in the Garvan Institute Biological Testing Facility. All experimental protocols were approved by the Garvan–St. Vincent’s Animal Ethics Committee.

**Adoptive transfers and immunizations**

B cells were purified with negative depletion using magnetic cell sorting and stimulated for 1 h with 10 μg/ml goat anti-mouse IgM (Jackson Immunoresearch). B cells (1-2 × 10^7) were injected i.v. into CD45.1 congenic mice, and splenocytes of recipient mice were collected 6 or 22 h later. The adoptive transfer system used to analyze responses of SWHEL B cells, mutant HEL3× protein and its conjugation to SRBCs have been described in detail previously (7, 18, 19).

**Flow cytometry**

Splenocytes were prepared, stained for surface molecules, and analyzed on a FACSCanto II (BD Biosciences) as described previously (7, 20). HEL3×-binding B cells were detected with 50 ng/ml HEL3× and purified by HEL9 hybridoma supernatant conjugated to Alexa Fluor 647 (Molecular Probes). The following Abs and reagents were used for cell staining: anti-CD184/ CXCR4-biotin (2B11; BD Biosciences), anti-CCR7-biotin (4B12; eBiosciences), anti-IgM-biotin (AF6-78; BD Biosciences), streptavidin-Pacific Blue (Invitrogen), anti-IgG1-FTTC (A85-1; BD Biosciences) anti-CD86-PE (GL1; BD Biosciences), anti-CD45.1-PE/Cy7 (A20; eBiosciences), anti-CD45.2-PerCP/Cy5.5 (104; eBiosciences), anti–CD35R/B220-PerCP/Cy5.5 (RA3-6B2; BD Biosciences), anti-CD45R/B220-Pacific Orange (RA3-6B2; Invitrogen), anti-CD38 Alexa Fluor 700 (90; eBiosciences), anti–IgD-Alexa Fluor 647 (11-26c.2a; BioLegend), MZ B cells with anti–CD23-PE (B3B4; BD Biosciences), anti–CD45.2-PerCP/Cy5.5 and goat anti-rabbit IgG-FITC (Southern Biotech). T cell areas were blocked with 30% horse serum. HEL-binding B cells were detected with hybridoma supernatant conjugated to Alexa Fluor 647 (Molecular Probes). The following Abs and reagents were used for cell staining: anti-CD184/ CXCR4-biotin (2B11; BD Biosciences), anti-CCR7-biotin (4B12; eBiosciences), anti-IgM-biotin (AF6-78; BD Biosciences), streptavidin-Pacific Blue (Invitrogen), anti-IgG1-FTTC (A85-1; BD Biosciences) anti-CD86-PE (GL1; BD Biosciences), anti-CD45.1-PE/Cy7 (A20; eBiosciences), anti-CD45.2-PerCP/Cy5.5 (104; eBiosciences), anti–CD35R/B220-PerCP/Cy5.5 (RA3-6B2; BD Biosciences), anti-CD45R/B220-Pacific Orange (RA3-6B2; Invitrogen), anti-CD38 Alexa Fluor 700 (90; eBiosciences), anti–IgD-Alexa Fluor 647 (11-26c.2a; BioLegend), MZ B cells with anti–IgM-FTTC (R6-60.2; BD Biosciences), and the marginal sinus with anti–IgM-FTTC (R6-60.2; BD Biosciences) followed by HRP-conjugated streptavidin and tyramide signal amplification-direct FITC-tyramide (PerkinElmer). Slides were analyzed on a Zeiss Axiosvert 200 microscope.

**Quantitative RT-PCR**

B cell populations were sorted into Trizol reagent (Invitrogen) and quantification of Ebi2 and Cyclin D2 expression was performed as described previously (7, 16). Expression of GAPDH was used for copy number normalization.

**Statistical analysis**

Statistical differences between means were assessed with one-way ANOVA and Tukey multiple comparison test.

Results

**EBI2 contributes to B cell organization in the spleen**

Previous studies have indicated that EBI2 deficiency has no obvious effect on the development of an organized lymphoid architecture (7, 8). Despite being expressed at a high level on both follicular and marginal zone (MZ) B cells (data not shown), EBI2 is not required for normal development of the follicular and MZ B cell compartments, and these populations localize normally on their respective sides of the marginal sinus in Ebi2−/− mice (Fig. 1A, 1B, Supplemental Fig. 1). Because CXCR5 has an essential role in the organization of B cells into follicles, we postulated that a function for EBI2 in this process might be revealed when the dominant effect of CXCR5 was removed. To test this theory, we generated mice deficient in both EBI2 and CXCR5 and compared the organization of B cells in the spleens of these mice to those from Cxcr5−/− mice. In Cxcr5−/− mice, B cells organize into rings around central T cell areas (2, 21) (Fig. 1C, Supplemental Fig. 1). In Ebi2−/−Cxcr5−/− mice, however, this B cell ring was less defined and showed intermingling of MZ and follicular B cells and the entry of many B cells into the T cell areas (Fig. 1C, Supplemental Fig. 1). Nevertheless, the frequencies of B cells with the CD21hiCD23loIgMloIgDhi MZ phenotype or the CD21lo CD23hiIgMhiIgDlo follicular phenotype were not significantly different in Cxcr5−/− and Ebi2−/−Cxcr5−/− mice (Fig 1D, 1E). In mice deficient in both CXCR5 and CCR7, the splenic architecture is further disrupted because T cells do not organize into T cell areas owing to a lack of CCR7 expression. In these mice, B cells aggregate into clusters (Fig. 1C, Supplemental Fig. 1) previously reported to form in areas rich in ER-TR7+ reticular fibroblasts and fibers (22). These B cell aggregates did not develop when expression of EBI2, CXCR5, and CCR7 was removed, with B cells in Ebi2−/−Cxcr5−/−Ccr7−/− mice exhibiting a relatively homogenous distribution throughout the spleen that reflected the absence of any defined nonlymphoid (red pulp) area (Fig. 1C, Supplemental Fig. 1). These results indicate that EBI2 directs B cell organization independently of CXCR5 and CCR7, most likely because its ligand is most concentrated within the reticular network. Despite the severely disrupted splenic architecture, Ebi2−/−Cxcr5−/−Ccr7−/− B cells developed into populations with a normal MZ and follicular phenotypes, and MZ B cells were slightly increased in Ebi2−/−Cxcr5−/−Ccr7−/− mice compared with WT and Cxcr5−/−Ccr7−/− mice (Fig. 1D, 1E). This finding is in marked contrast to Lta−/−, Lib−/−, and Lib−/− mice, in which the disorganization of the normal splenic structures, because of the inability of B cell lymphotixin-αβ to interact with stromal cell lymphotixin-β receptor, is accompanied by a complete loss of MZ phenotype B cells (23–25). Thus, the data presented in this study demonstrate that MZ B cells can develop and retain their phenotype through lymphotixin-mediated signaling, even in the absence of any recognizable MZ structure.

**EBI2 directly mediates B cell migration toward MZ bridging channels and peril follicular areas of the spleen**

To gain more insight into the mechanism by which EBI2 controls B cells chemotaxis, we assessed the migration of B cells from EBI2, CXCR5, and CCR7 single and compound gene knockout mice within intact, wild-type (WT) spleens. For this purpose, purified B cells were first activated for 1 h with anti-IgM Abs to cause of the inability of B cell lymphotoxin-α to interact with stromal cell lymphotoxin-β receptor, is accompanied by a complete loss of MZ phenotype B cells (23–25). Thus, the data presented in this study demonstrate that MZ B cells can develop and retain their phenotype through lymphotixin-mediated signaling, even in the absence of any recognizable MZ structure.
The migration pattern was similar to that observed for naive B cells. As previously shown, EBI2-deficient B cells injected into WT mice preferentially accumulated in the follicular dendritic cell-dense areas at the center of B cell follicles, and their localization in the periphery of follicles was reduced compared with WT B cells (7) (Fig. 2B). This result suggests that EBI2 actively attracts B cells to the outer areas of B cell follicles, but it could also be explained by EBI2 acting to inhibit CXCR5-mediated chemotaxis. To test this possibility, we compared the migration pattern of adoptively transferred Cxcr5−/− and Ebi2−/−Cxcr5−/− B cells. B cells deficient in CXCR5 were excluded from follicles and congregated specifically in MZ bridging channels (Fig. 2B). Additional removal of EBI2 expression disrupted this migration pattern, with Ebi2−/−Cxcr5−/− B cells localizing in T cell areas and to a minor extent in the red pulp (Fig. 2B). Thus, EBI2 constrained B cells to interfollicular zones and prevented them from localizing in T cell areas. In addition, whereas transferred Cxcr5−/−Ccr7−/− B cells were found to form clusters around the perimeter of B cell follicles, in particular in proximity of bridging channels, Ebi2−/−Cxcr5−/−Ccr7−/− B cells were evenly scattered throughout the red pulp and were not attracted to perifollicular areas (Fig. 2B). These findings indicate that EBI2 controls B cell localization in the spleen and is essential for normal B cell organization.
migration through its own direct action and not through modulating chemotactic signals delivered through CXCR5, CCR7, or both. Furthermore, these data clearly demonstrate that EBI2 directs B cell migration toward the outer edges of the splenic white pulp, in particular the follicular perimeter and the MZ bridging channels.

**EBI2 balances CCR7-mediated chemotaxis toward the T cell zone by directing B cells to the outer region of follicles**

CCR7 plays a central role in the migration of activated B cells. In particular, the increased expression of CCR7 resulting from acute BCR stimulation directs B cells toward the border of the follicle and the T cell zone (26) (Fig. 3A). Strikingly, activation of B cells that lack CCR7 not only fails to direct these cells to the T–B boundary region, but results in them migrating in the opposite direction, localizing proximal to the marginal sinus region in the outer area of B cell follicles (26) (Fig. 3A). To assess whether this alternate migration pattern in the absence of CCR7 is mediated by the activity of EBI2, we compared the localization of transferred Ccr7<sup>−/−</sup> and Ebi2<sup>−/−</sup> Ccr7<sup>−/−</sup> B cells 6 h after anti-IgM stimulation. B cells deficient in both EBI2 and CCR7 did not migrate toward either the T cell zone or the marginal sinus, but accumulated in the center of the B cell follicle (Fig. 3A), presumably because of the dominant activity of CXCR5. Thus, the migration of CCR7-deficient B cells to the outer follicle is confirmed to occur via EBI2-mediated chemotaxis.

It was apparent that the CCR7-mediated localization of WT and Ebi2<sup>−/−</sup> B cells proximal to the T cell zone 6 h after BCR stimulation (Fig. 3A) alters significantly over the subsequent 16 h. Thus, normalization of CCR7 expression by this point following anti-IgM treatment (Fig. 2A) results in both types of B cells returning to the follicle, WT B cells redistributing evenly across the follicle, and EBI2−/− B cells maintaining a more central distribution.
the follicle, and Ebi2−/− B cells clustering in the center (Fig. 2B). In contrast, Ccr7−/− and Ebi2−/−Ccr7−/− B cells persisted in the outer and central follicular regions respectively between 6 and 22 h after BCR stimulation (Fig. 3A, 3B). These results indicate that the role of EBI2 in regulating the migration of activated B cells within the follicle becomes dominant once the initial upregulation of CCR7 by BCR triggering has waned and the cells move away from the T–B boundary region and to the perimeter of B cell follicles adjacent to the MZ and to interfollicular areas (27, 28).

EBI2 antagonizes CCR7-induced migration in the early stages of Ab responses and directs activated B cells from the T–B boundary to the periphery of follicles

To verify the relative roles of EBI2 and CCR7 during the early phase of B cell responses, we assessed the migration pattern of Ebi2−/−, Ccr7−/−, Ebi2−/−Ccr7−/−, and WT B cells 6 h after transfer into WT CD45.1 congenic recipients. Immunofluorescence staining was performed as for Fig. 2. Pictures are representative of at least three mice. Insets show enlargements of the boxed areas. The relative distribution of transferred B cells in the outer follicle or in the follicle center is shown on the right. Splenic regions were defined as shown in Supplemental Fig. 2. Scale bars, 100 μm.

EBI2 antagonizes CCR7-induced migration in the early stages of Ab responses and directs activated B cells from the T–B boundary to the periphery of follicles

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FIGURE 3. EBI2 directs B cell migration to peripheral areas of follicles. A, Distribution of anti–IgM-stimulated polyclonal Ebi2−/−, Ccr7−/−, Ebi2−/−Ccr7−/−, and WT B cells 22 h after transfer into WT CD45.1 congenic recipients. B, Distribution of anti–IgM-stimulated polyclonal Ccr7−/− and Ebi2−/−Ccr7−/− B cells 22 h after transfer into WT CD45.1 congenic recipients. Immunofluorescence staining was performed as for Fig. 2. Pictures are representative of at least three mice. Insets show enlargements of the boxed areas. The relative distribution of transferred B cells in the outer follicle or in the follicle center is shown on the right. Splenic regions were defined as shown in Supplemental Fig. 2. Scale bars, 100 μm.

The downregulation of Ebi2 expression during T-dependent B cell responses is critical for establishing the GC reaction. Whereas Ebi2 is strongly downregulated in all GC B cells compared with naive B cells (7, 8, 15), it has been reported recently that LZ B cells express ~4-fold higher levels of Ebi2 than do DZ B cells (Fig. 5A) (16). This finding raises the possibility that differential expression of EBI2 might have a role in balancing the number or localization of LZ and DZ B cells or in other aspects of GC function. To test this possibility, we sought to analyze GC B cell differentiation and affinity maturation of Ebi2−/− SWHEL B cells during their response to HEL3x-SRBC within WT CD45.1 congenic recipients. Analysis of the response 10 d after immunization indicated that the proportion of cells with a LZ (CXR4low CD86high) as opposed to a DZ (CXR4high CD86low) GC B cell phenotype was slightly but consistently increased in the Ebi2−/− versus the WT SWHEL GC compartment (Fig. 5B, 5E). However, further analysis revealed that the frequency of cells with increased binding affinity for the immunizing Ag did not significantly differ between Ebi2−/− and WT GC B cells (Fig. 5B, 5F), indicating that the shift in DZ/LZ ratios associated with EBI2-deficiency did not have a major effect on affinity maturation. To test whether the kinetics of the GC response might be influenced by B cell-expressed EBI2, we next cotransferred Ebi2−/− (CD45.1−CD45.2+) and WT (CD45.1+CD45.2+) SWHEL B cells and followed their response to HEL3x-SRBC within the same recipient mice.
Under these conditions the magnitude of the GC responses derived from each population of donor B cells developed with very similar kinetics (Supplemental Fig. 3A) as did the appearance and rate of selection of GC B cells with high affinity for HEL3 (Fig. 5C).

In light of the EBI2-mediated functions revealed through our previous analysis of B cells with compound deficiencies in CXCR7 and EBI2 (Fig. 3 and 4), we tested whether a role for EBI2 in the GC response might be emphasized in the absence of CCR7 expression. GC responses established with Ebi2<sup>−/−</sup>Ccr7<sup>−/−</sup>SWHEL B cells showed a significant decrease in the DZ to LZ GC B cell ratio (Fig. 5D and E), similar to but more pronounced than was observed for Ebi2<sup>−/−</sup> SWHEL B cells. In addition, the Ebi2<sup>−/−</sup>Ccr7<sup>−/−</sup> SWHEL GC B cell population contained higher frequencies of low affinity cells in comparison with Ccr7<sup>−/−</sup> SWHEL GC B cells (Fig 5D and F). Ccr7<sup>−/−</sup> but not Ebi2<sup>−/−</sup>Ccr7<sup>−/−</sup> SWHEL B cells mounted enhanced GC responses (Supplemental Fig. 3B), but the localization within follicles of GCs arising from Ccr7<sup>−/−</sup> and Ebi2<sup>−/−</sup>Ccr7<sup>−/−</sup> SWHEL B cells was normal (data not shown). It appears, therefore, that the differential expression of EBI2 on DZ and LZ B cells influences the balance between these two GC subsets and that this might have a role in promoting affinity maturation.

**Discussion**

The movement and organization of B cells within follicles is controlled by the functions of CXCR5, CCR7 and EBI2, each mediating chemotaxis toward defined areas within secondary lymphoid organs. Through the production of compound gene knockout mice, we show that expression of all three of these receptors is required to direct B cells within the spleen both in the steady state as well as during immune responses. Expression of EBI2 is shown to direct B cell migration to perifollicular and interfollicular regions and to balance the migratory signals derived from lymphoid chemokines CXCL13 and CCL19/21 present in B cell follicles and T cell areas, respectively. The data presented in this study clearly demonstrate that the activity of EBI2 independently mediates chemotaxis of B cells toward the outer areas of...
FIGURE 5. Differential expression of EBI2 on DZ and LZ B cells influences GC organization and affinity maturation. SW<sub>HEL</sub> B cell of the indicated genotype were transferred into WT CD45.1 congenic recipients and challenged with HEL<sup>33</sup>-SRBCs. A, Quantitative PCR analysis of Ebi2 mRNA levels in sorted DZ (CXCR4<sup>hi</sup>CD86<sup>lo</sup>) and LZ (CXCR4<sup>lo</sup>CD86<sup>hi</sup>) WT SW<sub>HEL</sub> B cells 10 d after immunization. Separation of DZ and LZ populations was confirmed by analysis of cyclin D2 expression (Supplemental Fig. 3A). B, Frequencies of Ebi2<sup>−/−</sup> and WT SW<sub>HEL</sub> GC B cells with a DZ and LZ phenotype (upper panels) and accumulation of IgG1-switched GC B cells with increased HEL<sup>33</sup>-binding capacity (lower panels) 10 d after immunization. C, Kinetics of the generation of high-affinity cells within the IgG1<sup>+</sup> Ebi2<sup>−/−</sup> and WT SW<sub>HEL</sub> B cell populations. Ebi2<sup>−/−</sup> and WT SW<sub>HEL</sub> B cells were cotransferred into the same recipient and distinguished on the basis of their CD45.1/CD45.2 expression. D, Phenotypic analysis of Ccr7<sup>−/−</sup> and Ebi2<sup>−/−</sup>Ccr7<sup>−/−</sup> GC B cells on day 10 after immunization to determine frequencies of GC B cells with a DZ and LZ phenotype (upper panels) and generation of B cells with increased binding affinity for the immunizing Ag (lower panels). E, DZ/LZ ratio of GC B cells with the indicated genotype 10 d after immunization. F, Frequencies of high- and low-affinity SW<sub>HEL</sub> B cells within the IgG1<sup>+</sup> GC B cell population of the indicated genotype. Means ± SD of three to six mice are shown; results are representative of two similar experiments. Statistically significant differences are indicated with asterisks (*p < 0.05, **p < 0.01, ***p < 0.01).
B cell follicles rather than modulating CXCR5 or CCR7 signaling. However, it is possible that EBI2 might modulate other chemokine receptors or be involved in the regulation of mechanisms of B cell migration.

The data presented in this study indicate that a high chemokinic gradient of the newly identified EBI2 ligand (12, 13) is likely to be present in splenic bridging channels and at the perimeter of B cell follicles. In addition, our data indicate that EBI2 ligand activity is concentrated in the splenic reticular network. Thus, we suggest that the source of this activity might correspond to ERRT7α cells of the reticular network of the marginal zone or, alternatively, to immune cell populations residing in the marginal zone and bridging channels, such as CD4+ dendritic cells or MZ and marginal metallophilic macrophages.

The significance of the dimension to B cell migration added by EB12 is underscored by its importance in the positioning of B cells in the early stages of Ab responses. Early after BCR triggering, when B cells relocalize to the T–B boundary as a result of CCR7 upregulation, EBI2 expression helps distributing B cells along the T–B boundary and prevents them from penetrating T cell areas. As CCR7 levels subside, the activity of EBI2 counteracts CCR7-induced chemotaxis and mediates migration of activated B cells to the periphery of B cell follicles. These early steps in B cell migration mediated by EBI2 have been shown to be essential for the generation of extrafollicular plasmablasts and for establishing GC reactions (7, 8). The additional role for the low levels of EBI2 expressed in the GC described in this study indicate that this receptor also has an ongoing role in the control of T-dependent B cell responses.

The important role of EBI2 in regulating Ab responses is reflected in the association of its dysregulation in human autoimmune and neoplastic diseases, such as lupus, type 1 diabetes, chronic lymphocytic leukemia, and acute myeloid leukemia (30–33). Based on its function in regulating B cell responses, EBI2 dysregulation is also likely to be implicated in a least some of the 75% of cases of common variable immunodeficiency in which the cause of the Ab deficiency is unknown (34). Insights into the function and mechanism of action of EBI2 therefore represent important advances for a deeper understanding of the involvement of EBI2 in human diseases.

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Disclosures
The authors have no financial conflicts of interest.

References
Supplementary Figure 1. Enlarged images from Figure 1 showing the structure of follicles in the spleen of wild-type, Ebi2⁺, Cxcr5⁺, Ebi2⁺Cxcr5⁺, Cxcr5⁻Ccr7⁺ and Ebi2⁺Cxcr5⁺Ccr7⁻ mice.
Supplementary Figure 2. Definition of splenic regions for enumeration of transferred or HEL-binding B cells (green). B cell follicles are visualized in blue, T cell areas in red.
Supplementary Figure 3. (A) Increased expression of cyclin D2 in sorted LZ B cell population compared to DZ B cells confirms the separation of DZ and LZ B cells. (B) Kinetics of GC reaction of WT and Ebi2^−/− B cells following co-transfer into WT recipients and immunization with HEL^3x-SRBCs. (C) Frequencies of HEL^3x-binding SWHEL GC B cells of the indicated genotype on day 10 of their response to HEL^3x-SRBCs.