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Marginal Zone B Cells Regulate Antigen Capture by Marginal Zone Macrophages


The marginal zone (MZ) of the mouse spleen contains macrophages that express receptors that trap pathogens, including the scavenger receptor macrophage receptor with a collagenous structure and the C-type lectin specific intracellular adhesion molecule-grabbing nonintegrin receptor 1 (SIGN-R1). We previously reported that expression of SIGN-R1 was decreased in CD19-deficient mice. In this study, we demonstrate that SIGN-R1 is expressed on a subset of macrophage receptor with a collagenous structure (MARCO)+ macrophages. This subset is diminished when MZ B cells are absent due to either genetic developmental defects or following transient migration of B cells out of the MZ. When B cells return to the MZ, there is a delay in recovery of SIGN-R1–expressing macrophages. During this period, capture of Ficoll, which for the macrophages requires SIGN-R1, remains defective not only by the macrophages, but also by the B cells. Thus, MZ B cells regulate expression of molecules on macrophages that are important for trapping Ag, which, in turn, is required for Ag capture by the B cells. The Journal of Immunology, 2011, 186: 2172–2181.

The marginal zone (MZ) forms the outer boundary of the white pulp in the mouse spleen and contains macrophages and B cells that surround blood sinuses (1). The MZ surrounds the follicles that contain follicular B cells. The MZ plays a critical role in defense against pathogens that have entered the circulation. A specialized macrophage, the MZ macrophage (MZM), expresses receptors such as the C-type lectin specific intracellular adhesion molecule-grabbing nonintegrin receptor 1 (SIGN-R1), which binds polysaccharides such as those found on the outer wall of bacteria (2, 3), and the scavenger receptor macrophage receptor with a collagenous structure (MARCO) (4, 5). Without SIGN-R1, MZM fail to trap blood-borne Streptococcus pneumoniae, which results in increased mortality (6, 7). MZ B cells, in turn, capture these Ags in transfer from MZM (8, 9) and rapidly produce protective Abs without T cell help (10). In addition, MZ B cells transport Ags into the follicle, where they are picked up by follicular dendritic cells (FDC) and trigger adaptive immunity (11, 12).

In our previous studies, we found that SIGN-R1 expression was markedly diminished in mice that lacked CD19 (13). The results suggested that a primary defect in the differentiation of MZ B cells, due to absence of CD19, resulted in a secondary defect in MZM. These results refined earlier observations that absence of all B cells resulted in loss of MZM, as well as reports that loss of MZM produced a secondary defect in MZ B cells (14–16). However, the changes in the macrophages resulting from the absence of MZ B cells and the functional consequences of these changes remained to be defined.

In more recent studies to understand the effect of MZ B cells on MZM, we find that MZM are not homogenous. In normal mice, there are at least two types of MZM (MARCO+SIGN-R1+ and MARCO+SIGN-R1−) with different phenotypes and functions. Although MARCO+SIGN-R1+ MZM remain in the absence of MZ B cells, the MARCO+SIGN-R1− subset disappears. We demonstrate that this also occurs in mice that lack MZ B cells for reasons other than CD19 deficiency. Even transient absence of MZ B cells results in a decrease in the percentage of MARCO+ MZM that express SIGN-R1. The functional consequence is that MZM that lack expression of SIGN-R1 have reduced capacity to bind a model Ag, Ficoll. Without SIGN-R1+ MZM, MZ B cells also are unable to capture Ficoll. Thus, the proper differentiation and localization of MZ B cells is required for expression of receptors on macrophages that capture Ag for presentation to the B cells.

Materials and Methods

Mice

C57BL/6 mice were obtained from The Jackson Laboratory. The C57BL/6 CD19-knockout (KO; CD19cre) was as previously described (13). Notch2 conditional heterozygous KO mice (Notch2flx/+) were provided by Dr. John Kearney, with the permission of Dr. Tom Gridley (17). S1P1loxp/+ mice were provided by Dr. Proia (18). Both of these were crossed with CD19cre. For FTY720 treatment, B6 mice were injected i.p. with 25 µg LPS (Sigma-Aldrich). For trinitrophenyl (TNP)-Ficoll treatment, B6 mice were injected i.p. with 0.1 mg LPS (Sigma-Aldrich) and 10 µg TNP-Ficoll (Biosearch Technologies). To identify macrophages, 200 µg heat-killed, Alexa Fluor 488-conjugated Staphylococcus aureus bioparticles (Invitrogen) were injected i.v. 30 min prior to...
sacrifice of the mice. The University of Alabama at Birmingham Institutional Animal Care and Use Committee approved all mouse protocols. Adoptive transfer experiments were as previously described (13).

Abs, flow cytometry, and immunofluorescence

Abs to the following targets were purchased and, in some cases, conjugated to Alexa fluorochromes: C1qRp (AA4.1-allophycocyanin; eBioscience), IgM (II-41–FITC, BD Pharmingen; II-41–PE-Cy7, BD Biosciences), CD23 (B3B4-PE; BD Pharmingen), B220 (RA3-6B2–PerCP and allophycocyanin-Cy7, BD-Pharmingen), CD1d (1B1-FITC, BD Pharmingen), IgM (goat anti-mouse Alexa Fluor 555; Invitrogen), CD4 (Alexa Fluor 647; Caltag Laboratories), Siglec-1 (Moma-biotin or purified; BMA Biomedicals), SIGN-R1 (ER-TR9-biotin; BMA Biomedicals), mucosal addressin cell adhesion molecule-1 (MAdCAM-1; MECA-367–purified; BD Pharmingen), MARCO (Santa Cruz Biotechnology), and TNF (G235-PE from BD Biosciences). Single-cell suspension of spleen were prepared, stained, and analyzed by flow cytometry as previously described (13). For flow cytometric analysis of macrophages, 200 μg heat-killed, Alexa Fluor 488-conjugated *S. aureus* bioparticles (catalog number S-23371, Invitrogen) were injected i.v. 30 min prior to sacrifice of the mice. Spleens were harvested, and splenocytes were stained with MARCO, followed by goat anti-rat IgG-Alexa 647 (Invitrogen) and SIGN-R1–biotin, followed by streptavidin-Pacific blue (Invitrogen). Slides of spleen sections were prepared and analyzed as previously described (13). False colors are used to increase contrast using Leica confocal software (Leica Microsystems).

![FIGURE 1. Lack of SIGN-R1 on MARCO+ MZM in CD19ko mice.](image)

**A.** Spleen sections from wild-type and CD19ko mice were stained for MARCO (green) and SIGN-R1 (blue) on MZM, for IgM (red) on B cells, and for CD4 (purple) on T cells. Original magnification ×200. **B.** SIGN-R1 cells per area and MARCO cells per area in representative MZ from wild-type and CD19ko mice were measured using image analysis software. The ratio of SIGN-R1 cells and MARCO cells were calculated. **C.** Wild-type and CD19ko mice were injected i.v. with *S. aureus* bioparticles, and splenocytes were harvested and stained for SIGN-R1. Bioparticle-positive cells were analyzed for SIGN-R1 expression.
Except as stated otherwise, green represents signals for Alexa 488 staining, blue for Alexa 350, red for Alexa 555, and magenta for Alexa 647. ImageJ 1.37V (National Institutes of Health) was used to count MARCO⁺ and SIGN-R1⁺ cells in follicles. Ten follicles of three representative mice were chosen in each group. Particles of MARCO and SIGN-R1 were counted by ImageJ and divided by the area of follicle or perimeter of follicle (depicted by MAdCAM-1/MOMA-1 staining). Similarly, in FTY720 treatment experiments, there were three mice in each time group, and we analyzed 10 follicles in images from the spleen of each mouse. Particles of SIGN-R1 were counted by ImageJ and divided by perimeter of follicle. Every experiment was repeated twice.

**Statistics**

Statistical comparisons between groups were made using the two-tailed Student t test. All experiments included at least three mice per group. All data shown are representative of at least three replicate experiments.

**Results**

The SIGN-R1⁺ subset of MZM is absent in CD19ko mice

SIGN-R1 is often used as a marker of MZM. However, the nearly complete absence of SIGN-R1, which we previously reported in CD19ko mice, could represent either the absence of MZM, due to apoptosis or migration, or failure of MZM to express this molecule. To address this question, we costained spleens for MARCO, which is another marker for MZM, as well as for SIGN-R1. In wild-type mice, both MARCO and SIGN-R1 are present, and SIGN-R1 colocalizes with MARCO, but SIGN-R1 is only expressed on a subset of the MARCO⁺ cells (Fig. 1A, top panels). Qualitatively, it appears that there is greater expression of SIGN-R1 in areas where there are more MZ B cells. Thus, SIGN-R1 appears to be expressed on a subset of MZM and perhaps those in contact with MZ B cells.

In the CD19ko mice, the near absence of SIGN-R1 expression is as we reported previously (Fig. 1A, bottom panels). However, MARCO⁺ cells are still present. The double-positive SIGN-R1⁺ MARCO⁺ population is almost totally missing, whereas single-positive SIGN-R1⁻MARCO⁺ MZM remain. By quantitative image analysis, SIGN-R1⁺ MZM are decreased by >85% in CD19ko mice. MARCO⁺ MZM are decreased by 35% (Fig. 1B). Thus, the percentage of MARCO⁺ cells that also express SIGN-R1 is also dramatically decreased in CD19ko mice to 20% of that in wild-type mice.

To confirm the loss of SIGN-R1 expression on MZM in CD19ko mice, we developed a strategy to study MZM by flow cytometry. Previous studies show that MARCO on MZM is required for these cells to bind *S. aureus* (15). Mice were injected with labeled *S. aureus* bioparticles, sacrificed 30 min later, and spleen cells were analyzed for cells with bound or internalized *S. aureus* particles (Fig. 1C). With this technique, we again observe a decrease in the mean level of SIGN-R1 expression on cells from CD19ko mice as compared with wild-type mice, confirming the loss of SIGN-R1⁺ MZM in CD19ko mice. In addition, even though the frequency of bioparticle-positive cells is comparable, the absolute number of this population is only half in CD19ko compared with the wild-type mice.

**SIGN-R1 expression of MZM reflects MZ B cells in Notch2-heterozygous mice**

In CD19ko mice, not only are MZ B cells absent, but follicular B cells are decreased in number, and germinal centers are also defective. We previously showed, by adoptive transfer experiments, that when CD19ko mice are reconstituted by wild-type B cells, which restores the MZ B cells, then SIGN-R1⁺ MZM are reconstituted, indicating that the effect was due to an intrinsic defect in the B cells. However, this also leads to a reconstitution of normal follicular cells and germinal centers (data not shown). Thus, the previous results do not rule out effects of CD19 on cells other than MZ B cells, or effects on the MZ B cells that are specific to a defect in CD19, that lead to failure of differentiation of MZM. In contrast, Notch2⁻⁻ mice have normal follicular B cells and germinal centers. To determine whether the loss of SIGN-R1⁺ MZM represents a specific feature related to CD19 deficiency or is a more general defect related to the absence of MZ B cells, we studied Notch2 heterozygous mice. Conditionally targeted deletion of Notch2 in B cells results in the absence of MZ B cells and their precursors (19). Notch2⁻⁻ CD19⁻⁻ double-deficient mice lack MZ B cells, and, as expected, SIGN-R1⁺ MZM are also absent (Fig. 2A, top panels). Notch2 heterozygous mice have a more variable phenotype: some have few MZ B cells, whereas some have a frequency comparable to wild-type mice (19). Splenocytes from mice that were genetically conditionally heterozygous for Notch2 in B cells were screened by flow cytometry for the presence or absence of MZ B cells (representative results are shown for both types of mice in Fig. 2B) (CD19cre⁺ mice have normal MZ). Splenic sections from mice that were so identified were then analyzed histologically for expression of SIGN-R1 on MZM. When MZ B cells are present, SIGN-R1 is
expressed on MZM (Fig. 2A, middle panels). Mice that lack MZ B cells as a result of conditional heterozygous deficiency of Notch 2 lack expression of SIGN-R1 (Fig. 2A, bottom panels), similar to what is observed in CD19ko mice (Fig. 2A, top panels). This suggests that the reduced expression of SIGN-R1 on MZM is a general defect when MZ B cells are absent.

**Transient migration of MZ B cells results in transient loss of SIGN-R1 MZM**

The two models presented above lack MZ B cells on the basis of a genetically determined developmental deficiency. We asked what would happen in developmentally normal mice if MZ B cells were induced to migrate out of the MZ. Sphingosine-1-phosphate (S1P) 1 and S1P3, two receptors for S1P, are expressed at high levels on MZ B cells and control their localization in the MZ (20). Treating mice with FTY720, an antagonist to S1P, releases the MZ B cells from the MZ so that MZ B cells migrate into the follicles (20). B cells move out of the MZ as early as 4 h posttreatment. At that time point, SIGN-R1 expression is already significantly decreased compared with control mice, although the defect is not as profound as in mice with genetically determined absence of MZ B cells (Fig. 3A). Although MZ B cells are again present in the MZ at 24 h, SIGN-R1 expression is not fully restored until 96 h later, as demonstrated by quantitative image analysis (Fig. 3B). Thus, transient migration of MZ B cells out of the MZ results in transient loss of SIGN-R1 expression on MZM. In these mice, MZ B cells and MZM develop normally (in contrast to the mice with genetically determined absence of MZ B cells), but the continued presence of MZ B cells is required to maintain SIGN-R1 expression. The restored expression of SIGN-R1 on MARCO+ MZM appears in proximity to IgM-bright MZ B cells, again suggesting that MZ B cells probably trigger SIGN-R1 expression on macrophages via a cell–cell contact (replicating this in vitro is hampered by the rapid apoptosis of the MZ B cells in culture).

**Ag capture by MZM is impaired without MZ B cells**

SIGN-R1 is a C-type lectin that plays an important role in uptake of polysaccharides, which are major T-independent Ags on various
bacteria (6, 21). To test the hypothesis that without MZ B cells, and hence secondarily without SIGN-R1 + macrophages, the trapping of certain types of Ag would be impaired, we compared CD19ko and wild-type mice after i.v. injection of TNP-Ficoll. In spleen of wild-type mice, TNP-Ficoll is trapped in the MZ (Fig. 5A). In contrast, in CD19ko mice, although both MARCO+ and MOMA-1+ macrophages are present, trapping of TNP-Ficoll is significantly reduced. After recovery of SIGN-R1 expression in CD19ko mice following adoptive transfer of wild-type B cells, TNP-Ficoll capture is restored (Fig. 5B). In the CD19ko mice in Fig. 5B, note that the S. aureus bioparticles were trapped in the MZ, which indicates that functional MZM were still present, but these MZM could not capture TNP-Ficoll efficiently. In S1P1 conditional KO mice, which contain mature MZ B cells that are mislocated in the follicle but that lack SIGN-R1+ MZM, TNP-Ficoll could not be efficiently trapped in the MZ at short time periods (Fig. 5C). Thus, SIGN-R1–expressing MZM, which require the presence of MZ B cells, play a crucial role in capturing certain types of Ags, such as TNP-Ficoll, in the spleen. When MZ B cells are absent or have migrated out of the MZ, MZM lose the ability to take up these Ags.

Capture of Ag by B cells is defective in mice that lack SIGN-R1+ MZM

Ag trapped by MZM is then captured by MZ B cells and transported into follicles (12). We tested whether conditions that deplete B cells from the MZ and secondarily reduce expression of SIGN-R1 would alter capture of Ag by B cells. LPS, which rapidly induces migration of MZ B cells out of the MZ, was injected i.p. into wild-type mice, and spleen sections were analyzed for expression of SIGN-R1 and trapping of Ficoll by macrophages (Fig. 6) and for capture of Ficoll by B cells (Fig. 7). At day 1 after LPS administration, MZ B cells were absent from the MZ, and SIGN-R1 expression was greatly reduced. At day 2, MZ B cells began to repopulate the MZ, whereas SIGN-R1+ MZM were still absent. SIGN-R1 expression on MZM was only restored by days 7–9 (Fig. 6A, 6B). Thus, at day 2 after LPS injection, B cells have repossessed the MZ, but SIGN-R1 expression is still absent. To analyze Ag trapping when MZ B cells were present but SIGN-R1+ MZM were not, mice were injected with either LPS or PBS, as a control, and then 2 d later injected with TNP-Ficoll. Mice were sacrificed 30 min postinjection with TNP-Ficoll, and splenic B cells were analyzed by flow cytometry for captured TNP.
uptake of Ag was observed in MZ B cells of wild-type mice that had been treated with PBS 2 d before. In contrast, despite the fact that MZ B cells were present, as determined by histology (note IgM+ cells outside Siglec-1+ marginal metallophilic macrophages or for MARCO (green) or SIGN-R1 (green) on MZM), Seventeen days posttransfer of wild-type B cells into CD19ko mice, host mice were injected i.v. with TNP-Ficoll and S. aureus bioparticles and sacrificed 30 min later. Spleen sections were analyzed for TNP (blue), S. aureus bioparticles (green), and IgM (red). C, Wild-type and S1P1loxP/loxPCD19cre/+ mice were injected with TNP-Ficoll as in A. Spleen sections were stained for TNP (red), MAdCAM-1 (green) on marginal sinus lining cells, and IgM (blue) on B cells. Original magnification ×200 (A, B) and ×100 (C).

FIGURE 5. Defective Ag uptake in mice that lack SIGN-R1+ MZM. A, TNP-Ficoll was injected i.v. into wild-type and CD19ko mice, and 30 min later, mice were sacrificed, and sequential spleen sections were stained for TNP (blue) and either IgM (red) on B cells and for Siglec-1 (green) on marginal metallophilic macrophages or for MARCO (green) or SIGN-R1 (green) on MZM. B, Seventeen days posttransfer of wild-type B cells into CD19ko mice, host mice were injected i.v. with TNP-Ficoll and S. aureus bioparticles and sacrificed 30 min later. Spleen sections were analyzed for TNP (blue), S. aureus bioparticles (green), and IgM (red). C, Wild-type and S1P1loxP/loxPCD19cre/+ mice were injected with TNP-Ficoll as in A. Spleen sections were stained for TNP (red), MAdCAM-1 (green) on marginal sinus lining cells, and IgM (blue) on B cells. Original magnification ×200 (A, B) and ×100 (C).

Discussion
MZ B cells provide a critical connection between innate and adaptive immunity. On one hand, they are a defense against blood-borne Ags, such as bacteria, that bind both the Ag receptor and TLRs and thereby trigger rapid, T-independent differentiation of MZ B cells into short-lived plasmablasts. In addition, MZ B cells efficiently transport Ag into the follicle, where the Ag is transferred to FDC, which play a role in the germinal center, the heart of the adaptive humoral response. We had found that SIGN-R1–expressing macrophages are lost in the MZ of the spleen of CD19ko mice (13). We report in this study that this is not due to absence of CD19 per se, but rather reflects a requirement for the physical presence of MZ B cells in the MZ. Other genetic defects that result in an absence of MZ B cells (Notch2 deficiency), or even just displacement of MZ B cells out of the MZ (S1P1loxP/loxPCD19cre/+), have an effect comparable to that observed in the absence of CD19. Furthermore, even transient migration of MZ B cells out of the MZ following administration of LPS or FTY720 results in a rapid and substantial loss of SIGN-R1.

Data from this study, in conjunction with findings from others (15, 22), suggest that there are bidirectional physical and functional interactions that take place between MZ B cells and MZM that have important implications for the formation and maintenance of the MZ itself, as well as the function of individual cell populations within the MZ. Studies have demonstrated that B cells are critical for the formation and maintenance of the MZ, including the sinus lining metallophilic macrophages and MZM (14, 24). Conversely, it has been shown that MARCO+ MZM play an important role in the retention of MZ B cells via a process that involves direct contact between MARCO expressed on MZM and MZ B cells (15). The present study demonstrates that the
maintenance of SIGN-R1+ MZM requires the physical presence of MZ B cells in the MZ itself. This finding is significant because SIGN-R1–expressing MZM perform multiple, important functions that are not compensated for by other MZM populations lacking SIGN-R1 expression.

Most importantly, in CD19ko and S1P1loxP/loxPCD19cre/+ mice that lack B cells in the MZ, minimal amounts of the classic polysaccharide model Ag, Ficoll, are retained in the MZ after i.v. injection. Indeed, the disappearance of SIGN-R1 expression that follows even transient migration of MZ B cells out of the MZ results in a reduction in capture of Ficoll by any cell in the MZ. Reconstitution of MZ B cells by adoptive transfer of wild-type B cells into CD19ko mice rescues the ability of cells in the MZ to trap polysaccharide Ags. However, this effect does not involve the direct capture of Ag by the MZ B cells themselves, but it requires the presence of SIGN-R1–expressing MZM, which are maintained by the MZ B cells, to first trap the Ag. This conclusion is supported by the finding that initially after reconstitution of MZ B cells following adoptive transfer of WT B cells into CD19ko mice, or repopulation of MZ B cells after acute depletion, when MZ B cells are present but SIGN-R1–expressing macrophages are not, the ability to capture Ficoll remains defective. Indeed, Ficoll trapping only returns after SIGN-R1+ MZM reappear several days later. This is consistent with earlier reports in which blocking SIGN-R1 also prevents the capture of Ficoll in the MZ (25, 26).

Studies have now shown that SIGN-R1–expressing MZM perform critical functions in addition to the capture of polysaccharide Ags. SIGN-R1 binds C1q, leading to the formation of a classical C3 convertase that promotes complement activation. Indeed, it has been proposed that SIGN-R1 on MZM plays a dominant role in formation of the C3 convertase that functions as the primary complement fixation pathway for pneumococcal polysaccharides (7). Thus, SIGN-R1 expressed on MZM is likely to be important for generating complement components that promote bacterial opsonization and uptake in addition to playing a direct role in binding and uptake of bacteria. More recently, SIGN-R1+ MZM have been shown to bind sialylated IgG present in i.v. Ig, leading to the production of anti-inflammatory factors that promote the...
generation of FcγRIIb+ effector macrophages as opposed to inflammatory macrophages (27). Thus, maintenance of SIGN-R1 expression by MZ B cells may be important for preventing the initiation of inflammatory responses until such time as bacterial pathogens are encountered. The binding sites on SIGN-R1 for mannan and 2,6-sialylated Fc fragments of IgG are overlapping, and thus, there may be competition between different ligands for binding to SIGN-R1 that leads to distinct MZM responses. In such a situation, MZ B cells may enforce SIGN-R1 expression and an anti-inflammatory steady state that can be shifted to an active inflammatory state upon encounter with bacterial polysaccharide Ags.

These findings indicate that MZ B cells not only provide a link between the innate and adaptive immune response, they also function as regulatory cells that control the phenotype and function of other cells involved in the acute innate immune response. Capture of Ag is important for survival after bacteremia, even apart from any effect on Ab responses (6, 7). Thus, it appears that MZ B cells are regulating a function of MZM that is important to protect against lethal infection but that is independent of lymphocyte-mediated immunity.

The impaired Ag trapping due to loss of SIGN-R1 expression on MZM also leads to decreased ability of MZ B cells to bind polysaccharide Ags. As shown in Fig. 7, even when MZ B cells are present in the MZ, they capture little or no Ficoll when SIGN-R1+ MZM are absent. Thus, the SIGN-R1+ MZM are critical for the initial uptake of polysaccharide Ags, which can then be transferred to MZ B cells, thereby promoting the early innate-like humoral response associated with differentiation of MZ B cells into short-lived plasma cells. Interestingly, this applies not only to those B cells with a MZ B cell phenotype, but also to B cells in the same mice with a follicular phenotype. Thus, the regulation of SIGN-R1+ MZM by MZ B cells alters the (noncognate) capture of Ag by follicular B cells, presumably as a result of diminished Ag availability due to impaired trafficking of Ficoll by MZ B cells into the follicles, either directly or after transfer first to FDC and then to follicular B cells.

MZ B cells can acquire Ag from blood DC (28) and MZM; however, they seem not able to acquire Ag directly from blood pathogens, which indicates that MZ B play a role as a second line APC, following Ag trapping and transfer by macrophages or DC. This is also consistent with earlier reports in which blocking SIGN-R1 prevents the capture of Ficoll in the MZ (23). Our current results emphasize the bidirectional nature of the interaction, as the MZ B cells induce the expression of molecules required for trapping and transfer of Ag by the MZM.

Previous reports, from us and others, have addressed the question of Ab responses. The short-term Ab response (day 4), initiated predominantly by MZ B cells, is decreased in SIGN-R1ko mice or mice injected with anti–SIGN-R1 Ab (22, 23). The decreased short-term Ab response is consistent with the impaired Ab capture in the MZ in the absence of SIGN-R1+ MZM demonstrated in this study. Similarly, the titer of intermediate-term Ab responses (days 10–14) is comparable in CD19ko and SIGN-R1ko mice (6, 29). Additionally, we previously demonstrated that the distribution of CD11c+ dendritic cells is also altered in CD19ko mice that lack MZ B cells (13). In the absence of MZ B cells, the dendritic cells no longer concentrate in the bridging channels and instead are distributed circumferentially around the MZ. Thus, although the functional effect associated with the redistribution of CD11c+ DC has not been fully elucidated, this may represent a second mechanism by which MZ B cells regulate APC and thereby alter the adaptive immune response.
Although MZM were first characterized in the 1980s, they are still not well understood due to the lack of characteristic markers and the lack of appropriate in vitro assays to assess their function. MARCO, a scavenger receptor, and SIGN-R1, a C-type lectin receptor, are to date the most well-characterized surface proteins expressed by MZM (4, 30–32). Whereas most previous reports have assumed that MZM are a homogeneous cell population, our findings demonstrate that MZM are not homogeneous; instead, SIGN-R1+ MZM constitute a subset of MARCO+ MZM. Using both immunofluorescence microscopy and flow cytometry, MARCO+ MZM could be clearly divided into two populations based on the expression of SIGN-R1: SIGN-R1+MARCO+ macrophages and SIGN-R1−MARCO− macrophages. SIGN-R1+ MZM exhibit a distinct functional role that cannot be compensated for by SIGN-R1−MARCO− MZM. In mice lacking SIGN-R1+ MZM, even though MARCO+ MZM are still present and capable of taking up S. aureus bioparticles, the ability to capture Ficoll in an acute manner is lost. Whereas MZ B cells are critical for maintenance of SIGN-R1+ MZM, the loss of MZ B cells has only a modest effect on the overall MARCO+SIGN-R1− MZM population that is likely a reflection of the loss of the SIGN-R1+ subset.

The type of signal that MZ B cells provide to maintain SIGN-R1+ MZM could be in the form of a differentiation, retention, or survival signal, although it should be noted that these distinct types of signals are not mutually exclusive. With respect to delivery of a differentiation signal, MZ B cells may induce the expression of SIGN-R1 on any given MARCO+ MZM. Alternatively, SIGN-R1+ cells might represent a subset of MARCO+ MZM that constitute a unique macrophage lineage within the MZ that is differentially regulated by MZ B cells. In support of the latter hypothesis, it was observed that in mice lacking MZ B cells, and which lack SIGN-R1+ MZM, there is a diminution in the total number of MARCO+ cells that is proportional to the reduction in the number of SIGN-R1+ cells. This suggests that MZ B cells may in fact control the fate of a subset of MZM, because if MZ B cells were simply regulating the expression of SIGN-R1 on MARCO+ MZM in a stochastic manner, then one would not expect a change in the total number MARCO+ cells when MZ B cells are absent, which is not the case (Fig. 1B). In terms of the possibility that MZ B cells deliver a retention signal that keeps SIGN-R1+ MZM from migrating out of the MZ, SIGN-R1 expression appears to be limited to the MZ in the spleen. We have not been able to detect the migration of SIGN-R1+ MZM into other areas of the spleen or into the blood after depletion of B cells from the MZ, suggesting that a retention signal is less likely. In contrast, the available evidence suggests that MZ B cells may indeed provide a survival signal that is critical for maintenance of SIGN-R1+ MZM because the ability to detect SIGN-R1 expression is rapidly lost within 2 to 3 h after acute migration of MZ B cells, and as noted above, there is a decrease in the total number of MARCO+ MZM that is proportional to the loss of SIGN-R1+ cells after MZ B cells are induced to migrate out of the MZ. Although this could in theory be due to acute downregulation of SIGN-R1 expression on MZM, this is less likely because one would expect that repopulation of MZ B cells in the MZ would lead to an equally rapid upregulation of SIGN-R1 expression on MZM, which is not the case. Indeed, it was observed that reacquisition of SIGN-R1+ MZM required anywhere from 2–10 d after B cells repopulated the MZ depending on the experimental system being studied. This finding also supports the conclusion that MZ B cells may control the differentiation of a unique subset of MZM.

In summary, our findings demonstrate that MZ B cells are essential for maintenance of SIGN-R1+ MZM in the MZ of the spleen. Loss of SIGN-R1-expressing MZM dramatically affects the acute uptake of Ficoll-type polysaccharides not only by MZM, but also MZ B cells. Thus, we have established a cross-talk pathway between B cells and certain macrophages: on the one hand, MZ B cells are required to trigger the expression of SIGN-R1 on MZM; on the other hand, B cells can only capture certain Ag efficiently when MZM express SIGN-R1. This implies that B cells, through regulation of SIGN-R1 on macrophages, have a feedback effect on Ag presentation, similar to the effect of the two-way interaction of T cells and the dendritic cells that are the APC for T cells. Considering this cross-talk between MZ B cells and MZM, impaired Ag capture in mice without MZ B cells in earlier reports (9, 33) could be due to a defect of SIGN-R1+ MZM. Because SIGN-R1 expression is important for other functions associated with complement activation and regulation of the inflammatory state in the animal, understanding the molecular mechanisms by which MZ B cells regulate SIGN-R1 expression in the MZ is significant. Moreover, studies will need to be extended to humans to elucidate the role that MZ B cell populations play in terms of regulating the function of macrophage and dendritic cell populations, as there are structural and phenotypic differences that may alter the nature of the physical and functional interactions between these cell populations.

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Disclosures
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References


