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Cutting Edge: Selection of B Lymphocyte Subsets Is Regulated by Natural IgM

Nicole Baker and Michael R. Ehrenstein

Natural IgM has a wide range of actions in the immune system. Here we demonstrate that mice lacking serum IgM have an expansion in splenic marginal zone B cells with a proportionately smaller reduction in follicular B cells. The increase in the marginal zone-follicular B cell ratio (and an expansion in peritoneal B1a cells) is fully reversed by administration of polyclonal IgM, but not by two IgM monoclonals. Mice engineered to have a secreted oligoclonal IgM repertoire with an endogenous membrane IgM also exhibited a similar expansion of marginal zone B cells. We propose that natural IgM, by virtue of its polyreactivity, enhances Ag-driven signaling through the B cell receptor and promotes the formation of follicular B cells. These results demonstrate that natural IgM regulates the selection of B lymphocyte subsets.

Materials and Methods

Mice

The generation of Sμ− mice has been described previously (2). Mice were backcrossed onto C57BL/6 mice for six generations and kept under specific pathogen-free conditions. Litter-matched controls were used for all experiments.

Generation of Vμ1s-transgenic mice

The Vμ1 construct (gift from Dr. M. Neuberger, Laboratory of Molecular Biology, Cambridge, U.K.) encodes a VH (a allotype) gene that binds to anti-4-hydroxy-3-nitrophenenacetyl under control of a VH promoter (15). It was modified by restriction enzyme digestion so that the membrane exon of the transgene was deleted but the secretory exon remained intact (Fig. 2A). The resulting 11-kb construct Vμ1s was used for promonoclonal injection, and transgenic mice were generated by standard techniques. The mice were tested for the presence of the transgene by Southern blotting and PCR and were backcrossed six times onto C57BL/6 mice and subsequently onto Sμ− mice.

Flow cytometric analyses

Flow cytometric analyses were performed using standard techniques, stained cells were analyzed on FACScan, and files were plotted using CellQuest software (BD Biosciences, San Jose, CA). The MZ:FO ratio was calculated using absolute numbers of B cells in the CD21hiCD23lo FO compartment and the CD21hiCD23lo FO compartment.

Injections and immune responses

Sμ− and litter-matched controls were injected i.p. on six occasions at regular intervals for 2 wk with 200 μg/mouse of a purified monoclonal (either TEPC-183, unknown specificity (here designated monoclonal A) or MOPC-104E, specific for α1,3-glucose (here designated monoclonal B); Sigma-Aldrich, St. Louis, MO) or polyclonal IgM Ab (Rockland, Gilbertsville, PA). To induce a T-independent response, mice were immunized i.p. with 5 μg of phosphorylcholine-Ficoll (PC-Ficoll; Biosearch Technologies, Novato, CA) in PBS. Mice were bled at regular intervals during the 2 wk after the immunization.
ELISA
Ig and Ag ELISAs were performed as previously described (2) using anti-class- or allotype reagents (BD PharMingen, San Diego, CA). The myeloperoxidase ELISA was performed with a Capta myeloperoxidase ELISA kit (Trinity Biotech, Bray, Ireland).

Immunofluorescence analysis of tissue sections
Standard immunofluorescence staining procedures were used on frozen spleen sections stained with FITC-conjugated anti-mouse IgM and Texas Red conjugated anti-mouse IgD (BD PharMingen). Sections were viewed using a confocal microscope (Axiovert 100; Zeiss, Oberkochen, Germany).

Results
MZ B cells are expanded in Sμ− mice
Mature splenic B cells can be divided into MZ and FO B cells and can be distinguished by surface markers using FACS. MZ B cells (CD21high/CD23low) are expanded in 4-mo-old Sμ− mice, whereas FO B cells are reduced (Fig. 1A). Whereas the increase in MZ B cell numbers is 2.5-fold, the reduction in follicular B cells is less (a 25% reduction) based on proportion of absolute cell numbers for individual mice. Wild-type (WT) and Sμ− mice have the same number of splenic B220+ B lymphocytes (WT, 1.27 ± 0.26 × 107; Sμ−, 1.12 ± 0.17 × 107). This contrasts with the cellular changes observed after a T-independent response to PC-Ficoll where MZ B cell numbers increase in both WT and Sμ− mice (compare immunized with naive) whereas absolute FO B cell numbers are unchanged. In older mice (12 mo) the MZ:FO B cell ratio is increased in WT mice and the difference in MZ/FO B cell ratio between Sμ− and WT mice is less striking at this age (Fig. 1, A and B). Spleen sections taken from naive 4-mo-old Sμ− mice stained with anti-IgM/anti-IgD demonstrated a larger IgM staining in MZ area on the periphery of the follicle compared with WT controls, further demonstrating the expansion in MZ B cells in Sμ− mice (Fig. 1C). There is also an increase in the number of splenic CD21high/CD23low B cells which predominantly consist of newly formed B lymphocytes but would also contain splenic B1 cells. Although the numbers of splenic B1 cells are small compared with B2 cells, there is an increase in B1 cells (CD5−) in Sμ− mice (4.5 ± 0.7% within the splenic B lymphocyte gate) compared with WT mice (2.26 ± 0.3%), consistent with previous observations (1).

Polyclonal, but not monoclonal or oligoclonal, IgM fully reverses the MZ B cell expansion
To determine whether polyclonal IgM was critical in governing the B cell compartments, two approaches were used. The first was to generate a transgenic mouse line (Vμ1s, IgH α allotype) in which B lymphocytes secreted a μ H chain, but the construct was engineered so that no transgene expression was detectable on the B cell surface (Fig. 2A). These Vμ1s-transgenic mice were backcrossed onto C57BL/6 mice and subsequently onto Sμ− mice to test whether MZ expansion would be reversed. Consistent with the removal of the CμA exons, B lymphocyte membrane expression of the transgene was undetectable by FACS (Fig. 2B), but IgM (derived only from the transgene) was present in the serum of Sμ−/Vμ1s mice (Fig. 2C). These mice would therefore have a secreted oligoclonal repertoire generated by a number of L chains pairing with the Vμ1 chain. The second method of replacing IgM was to administer a polyclonal preparation of IgM or two IgM monoclonals i.p. every 3 days for 2 wk. The serum concentration of these preparations during the 2-wk period were identical (Fig. 2C).

The main conclusion from these series of IgM replacement experiments is that only the polyclonal IgM preparation returned the increased splenic MZ:FO B cell ratio found in Sμ− mice back to that observed in control mice as shown by FACS and tissue staining (Fig. 2, D–F). A much smaller reduction in the MZ:FO B cell ratio occurred after administration of monoclonal A. The increased IgM staining on the periphery of the follicle seen in Sμ− mice was restored to control levels by the administration of polyclonal IgM but not by the other monoclonals or in Vμ1s/Sμ− mice (Fig. 2F).

Effects of IgM reconstitution on B1 cells
Consistent with previous observations, peritoneal B1a cells were increased in Sμ− mice (1, 2). This expansion in B1a cells was reversed by polyclonal IgM and to a lesser extent by monoclonal A but not in those mice expressing the Vμ1s transgene or the mice that had received the monoclonal IgM B preparation (Fig. 3).

Polyclonal IgM is polyreactive
To determine whether the reactivity of the different IgM preparations was associated with their ability to influence MZ and B1 cells, binding to four different Ags was assayed. Analysis of the binding of these IgM preparations to the Ags tested confirmed the multireactivity of polyclonal IgM which bound all four Ags strongly and monoclonal A bound two Ags strongly, whereas monoclonal B bound only one Ag (Fig. 4). Analysis using HEP-2 cells for antinuclear Ab staining demonstrated that the polyclonal preparation and monoclonal A but not monoclonal B were positive.
FIGURE 2. Effects of IgM reconstitution on B cell subsets. A, Transgene construct of Vμ1s. The secretory μ tailpiece (tp) and the μ polyadenylation site are located within the Vμ1s construct; the μ constant region (CμM) and the μμ polyadenylation have been removed from the original Vμ1 construct and are shown for information only. B, FACS analysis of spleen cells taken from mice expressing the Vμ1s transgene using IgM allotype-specific markers. The IgM allotype of the Vμ1 transgene and the targeted Sμ− allele are α, whereas the BL/6 allotype is b. C, IgM serum concentration in Sμ−/μ1s mice and in mice that had received a polyclonal (poly) preparation or two monoclonal (mono A, mono B) preparations of purified IgM measured 2 days after the final injection of the different IgM preparations (three mice per group). Samples taken 1 or 2 days after each injection were not significantly different from results shown (data not shown). ○, IgMb allotype; □, IgMc allotype. D, Representative density plots to define proportion of MZ and FO cells using CD21 and CD23 as markers after administration of monoclonal A, monoclonal B, and polyclonal IgM (poly) and in Sμ−/μ1s mice. The density plots shown are gated on B220+ B cells. E, Histogram showing splenic MZ:FO B cell ratio calculated using absolute cell numbers in individual cell fractions as shown in D. MZ and FO were defined from FACS analysis as illustrated in Fig. 1A. Each bar represents the mean of six mice ± SD. F, Representative spleen sections derived from Sμ− mice treated with monoclonal B or polyclonal IgM stained with anti-IgM (FITC, green) and anti-IgD (Texas Red, red). All mice were between 3 and 4 mo old.

(data not shown). Thus, the Ag reactivity of the IgM preparations correlated with their ability to reverse the changes in B cell subsets found in Sμ− mice.

Discussion

The major finding of this work is that a selective deficiency of serum IgM leads to an increase in splenic MZ B cells with a proportionally smaller reduction in FO B cells. Furthermore, the fact that the increased MZ:FO B cell ratio is fully reversed by adding back polyclonal, but not monoclonal or oligoclonal, IgM suggests that a full repertoire of IgM molecules is required to regulate these B cell subsets. The difference between these preparations of IgM on splenic B cell subsets may be due to the multiple Ag reactivity of polyclonal IgM because this preparation bound to all the Ags tested and had the greatest effect on the MZ:FO B cell ratio. Monoclonal B had the least reactivity of the IgM preparations and no effect on the MZ:FO ratio. Administration of polyclonal IgM also reversed the expansion in peritoneal B1 cells previously documented (1, 2). Although there are a number of mechanisms that might explain the increased MZ:FO B cell ratio seen in Sμ−, as discussed below, we favor the hypothesis that IgM is acting as an adjuvant to deliver enhanced (self) Ag-driven signals to the BCR.

It has been proposed that the balance between splenic MZ and FO B cells is regulated by B cell signaling and that increasing clonal B cell signals favors the formation of FO over MZ B cells (10). The pentameric structure of IgM, in contrast to IgG, can potentially yield a high avidity for Ag despite a low Ag-specific affinity. Moreover, one molecule of IgM can activate complement, whereas two IgG molecules are required; this may lead to enhanced complement coating of selected self Ags when complexed to IgM, resulting in more efficient engagement of coreceptor signals (16, 17). Consequently, BCR signaling could be reduced and MZ B cells preferentially formed in the absence of IgM, despite normal levels of serum IgG. A similar process may apply to B1 cells, which are also sensitive to Ag drive (11), because polyclonal IgM also reversed the expansion of this B cell subset. The MZ:FO B cell ratio increases in mice as they age (this work and Ref. 18), which may be explained by defective signaling through the BCR which is also associated with aging (19), because MZ B cells are preferentially formed when BCR signals are reduced (10). Thus, the less striking difference between older Sμ− and control mice could be attributed to the already dampened BCR signaling associated with age.

The expansion in MZ B cell numbers in Sμ− mice may be explained by a number of mechanisms apart from B cell signaling. Mice bearing Ig chain transgenes with a variety of Ag specificities have an increased MZ B cell compartment. Because these mice have a restricted Ab repertoire, it has been proposed that the drive to make a complete repertoire increases MZ B cell numbers (6). Clearly, in Sμ− mice, the secreted IgM repertoire is absent whereas a full membrane IgM repertoire is present. If this proposed drive arises from the serum Ab repertoire then one would predict, as is observed here, that in Sμ− mice MZ B cell numbers would be increased and that polyclonal but not monoclonal IgM would reverse the changes. The transgenic mouse Vμ1s crossed onto the Sμ− mouse also has a restricted secreted IgM repertoire with a normal membrane BCR repertoire, supporting the notion that it is the secreted repertoire that is important in governing B1 and MZ B cell numbers. This line of reasoning, however, presumes that the serum IgG repertoire does not constitute a full Ab repertoire. A loss of diversity in the Ab repertoire is also observed in aging...
feedback mechanism that simply senses the IgM concentration. However, polyclonal IgM may have an advantage even in this situation if a putative FcR binds preferentially to the altered conformation arising out of IgM complexed with Ag. An FcR has been described, although not with these properties (21). However, a recent report has demonstrated that serum IgM is a ligand for CD19 (22), and B1 and MZ B cells are affected by alterations in CD19 expression (8, 23).

One apparent paradox of these observations is the short time (2 wk) required to reverse the MZ:FO ratio coupled with the evidence that MZ and FO B cells have a relatively long life span (24). This long life span, however, is somewhat dependent on the numbers of immature B cells entering the mature B cell pool. The finding that newly formed B cells increase in Sµ- mice may suggest either that these cells are being retained in this pool longer or that there are more immature B cells exiting from the marrow. It is known, and confirmed here, that MZ B cell numbers change during a 2-wk period after encountering T-independent Ags (6). Thus, changes in these B cell subsets could occur through a variety of mechanisms including alterations in cell proliferation or apoptosis, or by interchange between the MZ and FO B cell pools, although how IgM influences these processes remains to be investigated. It is tempting to speculate that IgM, through regulation of these B cell subsets, may have a role in suppressing the autoimmunity that occurs in mice strains where the MZ and B1 B cells are expanded.

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References


