CD22 Ligand Binding Regulates Normal and Malignant B Lymphocyte Survival In Vivo

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CD22 Ligand Binding Regulates Normal and Malignant B Lymphocyte Survival In Vivo


The CD22 extracellular domain regulates B lymphocyte function by interacting with α2,6-linked sialic acid-bearing ligands. To understand how CD22 ligand interactions affect B cell function in vivo, mouse anti-mouse CD22 mAbs were generated that inhibit CD22 ligand binding to varying degrees. Remarkably, mAbs which blocked CD22 ligand binding accelerated mature B cell turnover by 2- to 4-fold in blood, spleen, and lymph nodes. CD22 ligand-blocking mAbs also inhibited the survival of adoptively transferred normal (73–88%) and malignant (90%) B cells in vivo. Moreover, mAbs that bound CD22 ligand binding domains induced significant CD22 internalization, depleted marginal zone B cells (82–99%), and reduced mature recirculating B cell numbers by 75–85%. The CD22 mAb effects were independent of complement and FcRs, and the CD22 mAbs had minimal effects in CD22AA mice that express mutated CD22 that is not capable of ligand binding. These data demonstrate that inhibition of CD22 ligand binding can disrupt normal and malignant B cell survival in vivo and suggest a novel mechanism of action for therapeutics targeting CD22 ligand binding domains. The Journal of Immunology, 2006, 177: 3063–3073.

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D22 is a B cell-specific glycoprotein of the Ig superfamilies expressed on the surface of maturing B cells coincident with IgD expression (1, 2). Following either CD22 or BCR ligation, the tyrosine-phosphorylated cytoplasmic domain of CD22 recruits effector molecules that regulate BCR and CD19 signaling (3–11). In addition to regulating cytoplasmic signaling, CD22 may regulate cell-cell interactions through its seven extra-cellular Ig-like domains (4, 12, 13). Specifically, the two membrane-distal Ig-like domains of CD22 bind to α2,6-linked sialic acid-bearing (sialoglycoconjugate) ligands expressed by hemopoietic and nonhemopoietic cells and serum proteins (13–18). Molecules bearing potential CD22 ligands have been identified in vitro, which include CD22 itself, CD45RO, IgM, members of the Ly-6 family of glycoproteins, and other structurally diverse proteins and lipids (19–22). CD22 also interacts with itself in cis to form homomultimeric complexes (23). Although little is known regarding the in vivo relevance of CD22 ligand binding either in cis or trans, understanding this process is critical for interpreting CD22 regulation of B cell function.

In addition to regulating signal transduction through its cytoplasmic domain, CD22 regulates B cell development and function through ligand-generated signals. Studies with CD22AA and CD22Δ1-2 mice expressing gene-targeted CD22 molecules that do not interact with sialoglycoconjugate ligands suggest that CD22 engagement influences the development or maintenance of the marginal zone B cell population, BCR-induced proliferation, and cell surface CD22, IgM, and MHC class II expression on mature B cells, whereas normal BCR signaling and Ca2+ mobilization are not dependent upon ligand engagement (24). In agreement with a negative signaling role for CD22, B cells from CD22−/− mice generate augmented calcium (Ca2+) responses following BCR cross-linking and exhibit an IgMIIIaMHC class IIhigh phenotype that is characteristic of stimulated B cells (25–28). Remarkably, however, CD22−/−, CD22AA, and CD22Δ1-2 B cells each exhibit significantly enhanced turnover in vivo (24, 27, 28). Whether increased turnover is due to the absence of CD22 ligand-generated signals, B cell hyperresponsiveness in CD22−/− mice, or development/mature defects common to CD22−/−, CD22AA, and CD22Δ1-2 B cells remains unclear.

In vitro studies have also suggested roles for CD22 ligand binding in regulating B cell function. Interactions between CD22 and its α2,6-linked sialoglycoconjugate ligands have been suggested to mediate innate self recognition, leading to damped B cell autoreactivity (29). The sialic acid binding domains of CD22 have also been proposed to negatively regulate BCR signaling (30, 31). The Cy34 mAb reactive with the Lyb-8.2 allele of Cd22 does not measurably impair B cell function in vitro (2, 32–34). However, treatment of mice with polyclonal rabbit anti-Cd22 IgG reduces the number of mature recirculating bone marrow B cells, with no other effects observed (35). Abs that block human CD22 ligand engagement trigger potent BCR-independent signals in vitro that can lead to apoptosis (36–38). In vivo, a humanized Cd22 mAb has also been used clinically in the treatment of patients with B cell malignancies and autoimmune disease (39, 40) and is currently in phase-III clinical trials for the treatment of systemic lupus erythematosus (41). This therapeutic mAb binds CD22 outside of its ligand binding domains but based on in vitro assays may induce B cell depletion in vivo by Ab-dependent cellular cytotoxicity, induced CD22 phosphorylation/signal transduction, CD22 internalization, and/or other pathways (40, 42). Despite all of the above studies, the effects of selectively blocking CD22 ligand binding on normal human or mouse B cell survival and function in vivo have not been assessed, particularly under conditions in which B cell development proceeds normally.
The biology of CD22 ligand binding is not only important for understanding normal B cell regulation, but is critical for elucidating the mechanisms through which CD22-directed therapies function. To address these issues, a panel of mouse anti-mouse CD22 mAbs that do not inhibit, or inhibit CD22 ligand binding to varying degrees, were generated and assessed for their in vivo effects. mAbs that bind CD22 ligand binding domains caused striking reductions in the blood and marginal zone B cell compartments. Even more remarkable was the finding that CD22 mAbs that blocked CD22 ligand binding significantly inhibited normal and malignant B cell survival within tissues in vivo. Thus, CD22 ligand binding is required for the maintenance of mature B cells in the periphery.

Materials and Methods

**Mice**

CD22 $^{−/−}$, CD22Δ1-2, and CD22AA mice on a mixed B6/129 background were generated as described previously (24). C57BL/6, FcεRI $^{−/−}$ (B6.129S-FcεRI1Rav), and Rag1 $^{−/−}$ (B6.129S7-Rag1tm1Mom) mice were obtained from The Jackson Laboratory. FcεRI common γ-chain-deficient (Fcγ-γ-) and FcεRI common γ-chain-deficient (Fcγ-R-) mice (B6.129P2-FcγRγ−) were obtained from Taconic Farms. C3 $^{−/−}$ mice were obtained from Dr. M. Carroll (Center for Blood Research, Boston, MA) (43). Experiments were performed on 2- to 3-mo-old mice housed under specific pathogen-free conditions. All studies and procedures were approved by the Duke University Animal Care and Use Committee.

**CD22 mab generation**

The MB22-8, -9, -10, and -11 mAbs were generated and assessed for their ability to block CD22-directed MBC (14, 18). These mAbs were isolated from hybridomas producing mAbs specifically reactive with CD22-transfected mouse L cells, but not with untransfected cells, were subcloned twice with isotypes determined using a Mouse mAb Isotyping kit (Amersham). CD22 mAbs were purified using protein G Hi-Trap columns according to the manufacturer’s instructions (Amersham). MB22-8 and -9 mAbs were IgG1 Abs. MB22-10 and -11 were originally isolated as IgG2a mAbs; however, based on a study by Martin et al. (44), MB22-10 and -11 were reisotyped as IgG2c mAbs due to their C57BL/6 origin. Nonetheless, IgG2a- and IgG2c-specific reagents both show significant reactivity with MB22-10 and -11 mAbs. Because IgG2c-specific reagents were only recently made available, mouse IgG2a isotype controls and IgG2a-specific reagents were used in experiments. Reactivity of mAbs with membrane-proximal CD22 Ig domains was determined using CD22AA and CD22Δ1-2 mice as well as with CDεRI-transfected COS cells and immunofluorescence staining. MB22-11 was prepared using immobilized pepstatin (Pierce) in 20 mM sodium acetate buffer (pH 4.1), according to manufacturer’s instructions. Complete digestion was confirmed by reducing and nonreducing SDS-PAGE. Sterile protein G-purified CD22 and isotype control mAbs (10–250 g of mAb/200 ml of PBS) were injected i.v.

**Abs and immunohistochemistry**

Abs used included FITC-conjugated anti-mouse CD22 N terminus (Cy34.1, TIB163; American Type Culture Collection); FITC-conjugated I-A/E-MHC class II (clone M5/114.15.2; American Type Culture Collection); FITC-conjugated rat anti-mouse CD120 (clone 7C6g), PE-conjugated rat anti-mouse CD1 (clone 1B1), PE-conjugated rat anti-mouse CD19 (clone 1D3), and cytochrome-conjugated rat anti-mouse B220 (clone RA3-6B2), all obtained from BD Pharmingen; FITC-conjugated polyclonal goat anti-mouse IgM, PE-conjugated rat anti-mouse IgM (clone 1B4B1), PE-conjugated rat anti-mouse IgD (clone 11-26), PE-conjugated polyclonal goat anti-mouse IgG1, and IgG2a and FITC-conjugated anti-mouse IgM, all obtained from Southern Biotechnology Associates. For immunohistochemistry, 5-μm-thick spleen sections were fixed in acetone, blocked with 5% rabbit serum, and stained with FITC-conjugated anti-B220 mAb and PE-conjugated anti-CD11b mAb or AP-conjugated anti-mouse IgM (Southern Biotechnology Associates) and biotinylated anti-mouse CD23 (BD Pharmingen), followed by streptavidin-HRP (Southern Biotechnology Associates) with detection using 3,3′-diaminobenzidine substrate and Vector Blue AP substrate kits (Vector Laboratories). Fluorescent digital images were merged to indicate marginal zone B cell location.

Flow cytometry analysis

Blood leukocyte numbers were quantified by hemocytometry following red cell lysis, with cell frequencies determined by immunofluorescence staining with flow cytometry analysis. Single-cell leukocyte suspensions from spleen, bone marrow (bilateral femur), peritoneal lavage, and peripheral lymph node (bilateral inguinal) were isolated, and the erythrocytes were lysed in Tris-buffered 100 mM ammonium chloride solution. Leukocytes were then stained at 4°C using predetermined optimal concentrations of Abs for 30 min. For whole blood, erythrocytes were lysed after staining using FACS Lysing Solution (BD Biosciences). Ab binding was analyzed on a FACSscan flow cytometer (BD Biosciences) by gating on cells with the forward and side light scatter properties of lymphocytes. Nonreactive, isotype-matched Abs (Caltag Laboratories/BD Pharmingen) were used as controls for background staining.

**Adhesion and CD22-Fc binding assays**

For cellular adhesion blocking experiments, L cells stably transfected with mouse CD22 cDNA (mCD22-L) were preincubated with purified mAbs at 4°C for 30 min, washed twice with DMEM, and incubated with mouse spleenocytes for 30 min at 4°C as described previously (14, 18). Cells were then washed three times with DMEM, and the remaining spleenocytes were eluted with CD22-transfected L cells were counted under a microscope. Parental (CD22 −) L cells were used as a negative control. The CD22-Fc fusion protein was constructed by fusing the first three NH2-terminal extracellular Ig-like domains of murine CD22 to the Fc portion of human IgG1. CD22-Fc was cloned into pIREs-2EGFP (Clontech) and expressed in 293T cells. CD22-Fc (0.2 μg) was preincubated with CD22 mAbs (2 μg/ml) for 15 min at room temperature. CD22-Fc/CD22 mAb complexes were then incubated with 2.5 × 105 CD22−/− spleen cells in 100 ml of DMEM containing 10% FCS for 30 min on ice. CD22-Fc protein binding was assessed using PE-conjugated Fab′, goat anti-human IgG Abs (Fcy fragment-specific; Jackson ImmunoResearch Laboratories) with flow cytometry analysis.

**B cell turn over analysis (BrdU uptake)**

Mice were given drinking water containing 0.8 mg/ml BrdU for 7 days and sacrificed. Tissues were harvested, and single leukocyte populations were analyzed for BrdU incorporation. Briefly, cells were fixed in 0.5% paraformaldehyde, permeabilized with 3 N HCl containing 0.5% Tween 20, neutralized with 0.1 M dithiothreitale, stained with a FITC-conjugated anti-BrdU Ab (BD Biosciences) and a PE-conjugated anti-mouse B220 mAb (Caltag Laboratories), and analyzed by flow cytometry.

**Ca2+ responses**

Splenocytes were isolated at room temperature and suspended (1 × 107/ml) in RPMI 1640 (Sigma-Aldrich) containing 5% FCS and 10% HEPES (Life Technologies). The cells were loaded with 1 μM indo-1 AM ester (Molecular Probes) for 30 min at 37°C, labeled with FITC-conjugated anti-B220 mAb for an additional 20 min, washed, and suspended in warm medium at 2 × 106/ml for flow cytometry. Baseline indo-1 fluorescence emission intensities at 405 nm and 525 nm were collected for 1 min before the addition of Fab′, anti-IgM Abs (40 μg/ml), with fluorescence ratios (405 nm/525 nm) plotted for B220+ gated cells at 20-s intervals. Some samples were preincubated for 2 min with MB22-10 (20 μg/ml) before beginning sample collection. Increases in Ca2+ mobilization are depicted by increased indo-1 fluorescence intensity ratios after Ab treatment relative to the fluorescence intensity of untreated cells.

**B cell proliferation**

Splenic B cells were enriched by Thy1.2-magnetic bead depletion (Dynal Biotech) and labeled with CFSE according to the manufacturer’s instructions at a final concentration of 0.5 μM (Molecular Probes). A total of 1 × 106 B cells (∼90% B220+) was cultured in 0.5 ml of complete RPMI 1640 containing 10% FCS in 48-well flat-bottom plates. B cells were cultured for 4 days in medium alone, or in the presence of Fab′, anti-mouse IgM Abs (Cappel), LPS (Escherichia coli 0111:B4; Sigma-Aldrich), or hamster anti-mouse CD40 mAb (HM40-3; BD Pharmingen). Proliferation was assessed by the cellular loss of CFSE fluorescence intensity over successive rounds of division as assessed by flow cytometry. Dead cells were excluded by propidium iodide staining. Fifty thousand total events were collected per culture condition.

**Adoptive transfer experiments**

A total of 5 × 107 CFSE-labeled wild-type splenocytes was i.v. injected into wild-type C57BL/6 or Rag1 $^{−/−}$ mice. On days 2 and 9, mice received...
250 μg of MB22-10, MB22-8, IgG2a, or IgG1 control mAbs i.v. Tissues were harvested on day 14, with single-cell suspensions isolated and counted. CFSE<sup>+</sup> cells were analyzed for B220, CD19, IgM, IgD, CD22, CD4, and CD8 expression by flow cytometry. For A20 studies, 1 × 10<sup>7</sup> A20 cells were injected i.v. into Rag1<sup>−/−</sup> mice. Mice received 250 μg of MB22-10 or IgG2a control mAb i.v. on day 2. Mice were sacrificed on day 7, with splenocytes counted and CD19<sup>+</sup>B220<sup>+</sup> cells analyzed by flow cytometry.

Statistical analysis

All data are shown as means ± SEM. The Student’s <i>t</i> test was used to determine the significance of differences between population means.

**Results**

**Generation of anti-mouse CD22 mAbs that block ligand binding**

A panel of mouse anti-mouse CD22 mAbs was generated using B cells from CD22<sup>−/−</sup> mice and assessed for their ability to inhibit heterotypic CD22-mediated intercellular adhesion in vitro. Five different CD22 mAbs were selected that inhibited CD22 ligand binding to different degrees. The MB22-8 and Cy34.1 (IgG1) mAbs had little, if any, effect on CD22-mediated adhesion (<10%) when used at 1 or 10 μg/ml concentrations (Fig. 1A, data not shown). The MB22-9 (IgG1), MB22-10 (IgG2c), and MB22-11 (IgG2c) mAbs inhibited CD22-mediated adhesion by ~35, 60, and 90%, respectively, at 1–10 μg/ml mAb concentrations (Fig. 1, A and B, data not shown). The ability of each CD22 mAb to block mouse CD22-Fc fusion protein binding to CD22<sup>−/−</sup> mouse T and B splenocytes was also assessed in vitro. The MB22-8 mAb did not bind the CD22-Fc fusion protein and therefore had no effect on its binding to B or T cells. By contrast, the Cy34.1 and MB22-9 mAbs enhanced the binding of CD22-Fc fusion protein (5- to 13-fold) to both T (Fig. 1C) and B (Fig. 1D) cells, presumably due to CD22-Fc multimerization following mAb binding. The MB22-10 mAb enhanced CD22-Fc binding to B cells (3-fold) but had no effect on T cell binding, suggesting that it partially inhibited CD22-Fc binding in comparison with the Cy34.1 and MB22-9 mAbs. The MB22-11 mAb completely blocked CD22-Fc binding to both T and B cells. Collectively, these results indicate that the MB22-9, -7, -10, and -11 mAbs block CD22 ligand binding to increasing degrees, respectively, whereas the Cy34.1 and MB22-8 mAbs do not inhibit CD22 ligand binding.

These CD22 mAbs were also assessed for their ability to bind splenocytes from wild-type, CD22<sup>−/−</sup>, and CD22Δ1-2 (lacking two amino-terminal Ig-like domains) mice. The MB22-8 mAb bound wild-type and CD22Δ1-2 splenocytes but not CD22<sup>−/−</sup> splenocytes, whereas the Cy34.1, MB22-9, -10, and -11 mAbs bound wild-type but not CD22Δ1-2 or CD22<sup>−/−</sup> splenocytes (Fig. 1E, data not shown). The MB22-9, -10, and -11 mAbs bind a similar region of CD22 because MB22-11 mAb binding was completely blocked by prior MB22-10 mAb binding and partially blocked by MB22-9 mAb binding (data not shown). By contrast, none of the MB22 mAbs inhibited Cy34.1 mAb binding. Thus, among the MB22 mAbs binding to the two amino-terminal ligand binding domains of CD22, the MB22-10 and MB22-11 mAbs exhibited the highest levels of blocking activity in vitro.

**CD22 ligand binding domain mAbs deplete blood and marginal zone B cells in vivo**

The effects of CD22 mAb treatment on B cell numbers were assessed in vivo. Although MB22-8 mAb treatment did not significantly affect blood B cell numbers, the Cy34.1, MB22-9, -10, and -11 mAbs reduced B cell numbers by ~75–85% within 3–10 days (Fig. 2A–C). T cell numbers were not affected (data not shown). The degree and duration of peripheral blood B cell depletion was dependent on mAb dose, as shown for the MB22-10 mAb (Fig. 2B). Reductions were comparable for 100–250-μg doses over a 2-wk period, with B cell numbers recovering earlier with 100-μg doses. A 25-μg dose of MB22-10 mAb gave moderate depletion, whereas 10 μg had little effect. The frequency of mature recirculating B220<sup>high</sup>IgD<sup>+</sup> B cells in the bone marrow was significantly reduced by all CD22 mAbs, including the MB22-8 mAb (Fig. 2D). However, no changes were observed in the frequencies of pre/pro-B or immature B cells (data not shown), which do not express or express low levels of cell surface CD22, respectively. Spleen and peritoneal cavity B cell numbers were unchanged 7 days after CD22 mAb treatment (Fig. 2E, data not shown). Lymph node B cell numbers were reduced slightly following MB22-9 and -10 treatments, with only the MB22-11 mAb causing significant reductions (Fig. 2F). Thus, CD22 mAb treatment predominantly reduced mature bone marrow and blood B cell numbers.
The effect of CD22 mAbs on the marginal zone B cell population was assessed in wild-type mice. The Cy34.1, MB22-9, -10, and -11 mAbs depleted significant numbers of CD1d^{high}/CD21^{high}/B220^{+} marginal zone B cells as determined by flow cytometry (Fig. 2, G and H), whereas the MB22-8 mAb did not have a significant effect (Fig. 2H).

Notably, marginal zone B cell numbers had begun to replenish 3 wks following a single 250-μg MB22-10 mAb injection (2.4 × 10^{5}/mouse; n = 2) compared with control mAb-treated littermates (3.8 × 10^{5}/mouse; n = 2). In vivo IgM^{bright}/CD23^{−} or B220^{+}/CD1d^{bright} marginal zone B cell depletion was confirmed by immunohistology following MB22-10 (Fig. 2) and MB22-11 (data not shown) mAb treatments. Thus, mAb binding to the CD22 ligand binding domains inhibited circulating and marginal zone B cell survival or localization.

**CD22 ligand-blocking mAbs increase B cell turnover in vivo**

Although CD22 mAb treatment did not significantly alter total B cell numbers in peripheral tissues, striking alterations in B cell turnover rates were observed 7 days following mAb treatment. No significant differences in B cell turnover were found among MB22-8, Cy34.1, and MB22-9 mAb treatments (Fig. 3A). However, blood, spleen, and lymph node B cell turnover was significantly higher in mice treated with the CD22 ligand-blocking mAbs, MB22-10 and MB22-11, in comparison to nonblocking mAbs. The relative frequencies of BrdU^{−} (cycling) blood, spleen, and lymph node B220^{+} cells were increased by as much as 4-fold in MB22-10 and MB22-11 mAb-treated mice compared with control mAb-treated mice 7 days following BrdU administration. Both the MB22-10 and MB22-11 mAbs had significant effects on the numbers of BrdU^{+}-labeled and unlabeled blood, lymph node, and spleen B cells (Fig. 3B). The total numbers of BrdU^{+} (non-cycling) spleen and lymph node B cells were significantly decreased by MB22-10 and -11 treatment, whereas numbers of BrdU^{+} B cells within these tissues increased or stayed the same. Similarly, CD22AA and CD22^{−/−} mice also had 2- to 3-fold increased frequencies of BrdU^{+} B cells in the bone marrow, blood, and spleen compared with littermates with identical genetic backgrounds (Fig. 3, C and D), as described previously (24, 27, 28). These results demonstrate that inhibiting CD22 ligand binding alters normal B cell turnover in the periphery.

**CD22 ligand-blocking mAbs inhibit normal and malignant B cell survival in vivo**

To directly address whether blocking CD22 ligand binding interfered with peripheral B cell survival, CFSE-labeled splenocytes were transferred into wild-type littermates and monitored for their ability to survive in vivo following CD22 mAb treatment. After 2 wk, the frequency and number of adoptively transferred (CFSE^{+}) B cells recovered from the blood, bone marrow, lymph nodes, and spleens of MB22-10 mAb-treated mice were decreased by 4- to 15-fold compared with control mAb-treated mice (Fig. 4, A and B). No significant differences were found in CFSE^{−} T cell numbers (Fig. 4B). Moreover, the ratios of CFSE^{+} B cells:CFSE^{−} T cells within these tissues were decreased by 4- to 19-fold following MB22-10 mAb-treatment (Fig. 4C). Similar results were observed 1 wk following MB22-10 mAb treatment (data not shown). By contrast, MB22-8 mAb treatment had no effect on the ratio of CFSE^{+} B cells:CFSE^{−} T cells in lymph nodes or spleen and only decreased this ratio by 1.6-fold in blood and bone marrow (Fig. 4D). Thus, blocking CD22 ligand binding significantly reduced mature B cell survival in vivo.

To assess whether CD22 similarly regulates the survival of malignant B cells, A20 lymphoma cells were transferred into Rag1^{−/−} mice and examined for their ability to survive following CD22 mAb treatment. The frequency and number of A20 lymphoma cells recovered from spleens of MB22-10 mAb-treated
mice was decreased by ~90% compared with control mAb-treated littermates (Fig. 4E). Thus, CD22 ligand binding regulates both mature and malignant B cell survival in the periphery.

**CD22 mAb effects on B cell phenotypes in vitro and in vivo**

Because blocking CD22 ligand binding increased B turnover in vivo (Fig. 3, A and B), the effects of CD22 mAbs on B cell phenotypes were assessed. All CD22 mAbs significantly reduced CD22 expression on spleen B cells during 20-h in vitro cultures (Fig. 5A: MB22-8, 45 ± 8%; Cy34.1, 69–70%; MB22-9, 82 ± 3%; MB22-10, 64 ± 6%; and MB22-11, 54 ± 8%, compared with CD22 mean fluorescence intensities (MFIs)3 for control cultures; n ≥ 3 experiments for MB22 mAbs, n = 2 for Cy34.1). Comparable results were obtained for shorter (1–2 h) incubation periods (data not shown). CD22 mAb binding to B cells from CD22AA mice also induced similar reductions in cell surface CD22 expression (Fig. 5B: MB22-8, 51 ± 10%; Cy34.1, 60–66%; MB22-9, 70 ± 7%; MB22-10, 55 ± 14%; and MB22-11, 67 ± 6%; n ≥ 3 experiments for MB22 mAbs, n = 2 for Cy34.1). Similar results were also obtained using B cells from FcγRIIB−/− mice (data not shown). In vitro CD22 mAb treatment had minimal effects on cell surface IgM, IgD, or CD19 expression (data not shown). Thus, all CD22 mAbs induced significant receptor internalization in vitro regardless of FcγRIIB expression or in the case of CD22AA B cells, prior to CD22 ligand engagement.

In vivo, MB22-9, -10, and -11 mAbs treatments significantly reduced cell surface CD22 expression on B cells to nearly undetectable levels in the spleen, blood, bone marrow, lymph node, and peritoneum as determined by Cy34.1 mAb staining 7 days after treatment (Fig. 5C, data not shown). CD22 expression was similarly reduced on adoptively transferred A20 cells remaining after MB22-10 treatment (Fig. 4E, data not shown). Reduced CD22 expression was likely due to CD22 internalization because the MB22-9 and -10 mAbs were not detected on the cell surface by isotype-specific secondary Abs (Fig. 5D, data not shown). MB22-8 mAb treatment only resulted in a 30 ± 3% reduction in Cy34.1 mAb staining (Fig. 5C), and residual cell surface bound MB22-8 mAb was often detected 7 days postinjection (Fig. 5D). Similarly, Cy34.1 mAb treatment resulted in the partial loss (78 ± 2%) of CD22 expression with residual Cy34.1 mAb also detected on the cell surface. Unlike the MB22-9 and MB22-10 mAbs, residual cell surface MB22-11 mAb was also sometimes observed on tissue B cells 7 days after treatment. Importantly, restoration of circulating B cell numbers coincided with the appearance of CD22− B cells (data not shown).

In vivo CD22 mAb treatment also induced significant reductions in cell surface IgM (40–50%) and CD19 (20–60%) expression by spleen, blood, lymph node, and peritoneal B cells (Fig. 5, E and F; data not shown). CD21 expression was also reduced by 30–60%. In comparison, IgD expression was only slightly decreased (~5–30%), whereas MHC class II expression was only slightly enhanced (~20%) on tissue B cells following MB22-10 mAb treatment (data not shown). CD1d and B20 expression were unaffected by CD22 mAb treatment (Fig. 5E and data not shown). Similar to the B cells remaining in wild-type mice after CD22 mAb treatment, small numbers of adoptively transferred CFSE+ B cells remained in mouse tissues following MB22-10 mAb treatment (Fig. 4, A–C). The residual B cells were B220+/IgD+, expressed significantly lower levels of CD19 and IgM, and were negative for CD22 expression (Fig. 5, G and H). Thus, the treatment of mice with mAbs that bind CD22 ligand binding domains either enriched for residual B cell populations expressing a unique phenotype or induced significant reductions in the expression of multiple regulatory surface molecules, including CD22, IgM, CD19, and CD21.

**CD22 mAb effects in CD22AA mice**

Whether CD22 mAbs exhibit their biological effects by inhibiting ligand-induced signals or by removing CD22 from the cell surface was assessed using CD22AA mice that express CD22 receptors incapable of ligand binding. In vivo, MB22-10 mAb treatment down-modulated B cell CD22 expression to undetectable levels in both wild-type and CD22AA mice (Fig. 6A). However, in contrast

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1 Abbreviation used in this paper: MFI, mean fluorescence intensity.
Whether CD22 mAb treatment in vivo affected BCR-induced B cell linking, whereas CD22 isotype control mAb-treated mice; (Fig. 6D, middle panel). B cells from CD22AA mice treated in vivo with MB22-10 mAb also generated augmented Ca\(^{2+}\) responses (Fig. 6D, right panel), likely due to the loss of cell surface CD22 (Fig. 6A). Wild-type B cells incubated with MB22-10 mAb in vitro for 2 min before BCR stimulation generated augmented Ca\(^{2+}\) responses (Fig. 6D, middle panel). In contrast, in vitro MB22-10 mAb pretreatment did not alter Ca\(^{2+}\) responses in CD22AA B cells (Fig. 6D, right panel). Finally, B cells isolated from MB22-8 mAb-treated mice had normal IgM-induced Ca\(^{2+}\) responses, whereas B cell preincubation with MB22-8 mAb before BCR stimulation resulted in augmented Ca\(^{2+}\) responses (data not shown). Thus, mAb engagement of CD22 may inhibit CD22 negative regulation independent of interfering with CD22 ligand binding.

CD22 mAb treatment had no effect on B cell survival during in vitro cultures and did not induce B cell proliferation or significantly affect anti-IgM- or LPS-induced proliferation (Fig. 6D, data not shown). However, the MB22-11 mAb synergized with CD40 mAbs to induce robust B cell proliferation. The MB22-8 mAb did not significantly augment proliferation, whereas the MB22-9 and MB22-10 mAbs had intermediate effects on CD40-induced proliferation. B cells isolated from MB22-10 and isotype control mAb-treated mice exhibited identical proliferative responses in vitro (Fig. 6F), unlike B cells from CD22+/− mice with impaired proliferation in response to IgM cross-linking and augmented responses to CD40 stimulation (25, 45). CD22 expression levels on these B cells approached normal levels 24 h after culturing either due to B cell maturation in vitro or recovery from CD22 mAb-induced internalization (data not shown). Nonetheless, in vivo CD22 mAb treatment did not induce CFSE\(^{−}\) B cell proliferation in either wild-type or Rag1−/− mice (Fig. 4A, data not shown). Thus, although CD22 mAb treatment resulted in augmented BCR-induced Ca\(^{2+}\) responses in vitro, anti-IgM Ab-induced B cell proliferation remained normal.

**CD22 mAbs deplete B cells independent of FcR and complement**

To further verify that CD22 mAbs altered B cell survival in vivo by interfering with CD22 ligand binding, the effects of MB22-10 mAb treatment were assessed in C3−/− mice deficient in complement-dependent cytotoxicity, and in FcγRIIB−/− and FcγRIγ−/− mice with enhanced or blocked Ab-dependent cellular cytotoxicity, respectively. FcγRI, FcγRIII, and FcγRIV ligand binding α-chains associate with a common stimulatory γ chain (FcγRγ) that is required for FcγR assembly and triggering of effector functions, including phagocytosis by macrophages and cytotoxicity by NK cells (46–48). MB22-10 mAb administration significantly reduced blood and marginal zone B cells, and mature recirculating bone marrow B cells after 7 days in C3−/−, FcγRIγ−/−, and FcγRIIB−/− mice compared with control mAb-treated littermates (Fig. 7, A–C, data not shown). MB22-10 mAb treatment also significantly reduced B cell CD22, CD19, and IgM expression in C3−/−, FcγRIγ−/−, and FcγRIIB−/− mice (Fig. 7, A–C, data not shown). Blood and marginal zone B cell numbers were also significantly reduced following treatments of wild-type mice with MB22-11 mAb F(ab′)\(_2\) (Fig. 7D). Treatment of mice with intact MB22-11 mAb yielded greater reductions, especially 6 days following mAb treatment, but this difference is likely due to the short in vivo half-life of F(ab′)\(_2\). CD22, CD19, and IgM expression levels were

**CD22 mAb effects on Ca\(^{2+}\) responses and proliferation**

Whether CD22 mAb treatment in vivo affected BCR-induced Ca\(^{2+}\) mobilization or proliferation was assessed in vitro. CD22AA B cells generated normal Ca\(^{2+}\) responses following IgM cross-linking, whereas CD22−/− B cells generated augmented Ca\(^{2+}\) responses (Fig. 6D, left panel), as demonstrated previously (24–28). Ca\(^{2+}\) responses of B cells from MB22-10 mAb-treated wild-type mice were augmented and delayed following IgM cross-linking compared with control mAb-treated mice, perhaps due to decreased CD22 and CD19 expression (Fig. 6D, middle panel). B cells from CD22AA mice treated in vivo with MB22-10 mAb also generated augmented Ca\(^{2+}\) responses (Fig. 6D, right panel), likely due to the loss of cell surface CD22 (Fig. 6A). Wild-type B cells incubated with MB22-10 mAb in vitro for 2 min before BCR stimulation generated augmented Ca\(^{2+}\) responses (Fig. 6D, middle panel). In contrast, in vitro MB22-10 mAb pretreatment did not alter Ca\(^{2+}\) responses in CD22AA B cells (Fig. 6D, right panel). Finally, B cells isolated from MB22-8 mAb-treated mice had normal IgM-induced Ca\(^{2+}\) responses, whereas B cell preincubation with MB22-8 mAb before BCR stimulation resulted in augmented Ca\(^{2+}\) responses (data not shown). Thus, mAb engagement of CD22 may inhibit CD22 negative regulation independent of interfering with CD22 ligand binding.

CD22 mAb treatment had no effect on B cell survival during in vitro cultures and did not induce B cell proliferation or significantly affect anti-IgM- or LPS-induced proliferation (Fig. 6D, data not shown). However, the MB22-11 mAb synergized with CD40 mAbs to induce robust B cell proliferation. The MB22-8 mAb did not significantly augment proliferation, whereas the MB22-9 and MB22-10 mAbs had intermediate effects on CD40-induced proliferation. B cells isolated from MB22-10 and isotype control mAb-treated mice exhibited identical proliferative responses in vitro (Fig. 6F), unlike B cells from CD22+/− mice with impaired proliferation in response to IgM cross-linking and augmented responses to CD40 stimulation (25, 45). CD22 expression levels on these B cells approached normal levels 24 h after culturing either due to B cell maturation in vitro or recovery from CD22 mAb-induced internalization (data not shown). Nonetheless, in vivo CD22 mAb treatment did not induce CFSE\(^{−}\) B cell proliferation in either wild-type or Rag1−/− mice (Fig. 4A, data not shown). Thus, although CD22 mAb treatment resulted in augmented BCR-induced Ca\(^{2+}\) responses in vitro, anti-IgM Ab-induced B cell proliferation remained normal.
FIGURE 5. Effects of CD22 mAb treatments on B cell phenotypes. A and B, CD22 internalization following incubation with CD22 mAbs in vitro. Fluorescence intensity of CD22 on purified B cells from wild-type (A) or CD22AA (B) mice incubated in medium (thick solid line) or with CD22 mAbs (20 μg/ml; thin solid line) for 20 h. Cells were stained with CD22 mAbs or isotype-matched control (dashed line) mAbs (10 μg/ml). In all cases, mAb staining was visualized using PE-conjugated isotype-specific secondary Ab with flow cytometry analysis. MB22 mAb results represent those obtained in ≥3 experiments. C–F, Splenocytes were assessed for CD22 (C and D) and IgM, IgD, CD19, CD21, and CD1d (E and F) expression 7 days following mAb injection by immunofluorescence staining with flow cytometry analysis. C, CD22 expression in mice treated with MB22 mAbs (thick lines) or isotype control (thin lines) mAbs assessed by Cy34.1 mAb staining. FITC-conjugated MB22-8 was used to assess CD22 expression in Cy34.1-treated mice. D, Cell surface CD22 mAb binding in mice treated with CD22 (thick lines) or isotype control (thin lines) mAbs assessed using isotype-specific secondary Abs. Results represent those obtained in ≥3 experiments. E, Cell surface molecule expression in mice treated with MB22-10 (thick lines) or isotype control (thin lines) mAbs. F, Mean IgM, IgD, CD19, and CD21 expression levels relative to wild-type controls (±SEM) are indicated for mice treated with CD22 or isotype control mAbs (n = 3 mice per treatment). *, Mean MFI values were significantly different from isotype control mAb-treated mice; p < 0.05. G and H, Fifty million CFSE-labeled wild-type splenocytes were i.v. injected into wild-type mice. On days 2 and 9, mice received 250 μg of MB22-10 (n = 4) or IgG2a control mAb (n = 4). Tissues were harvested on day 14, and CFSE<sup>+</sup> cells were analyzed for CD22, CD19, IgM, and CD22 expression by flow cytometry. G, CD19 expression by CFSE<sup>+</sup> spleen cells, and IgM and CD22 expression by CFSE<sup>+</sup>B220<sup>+</sup> gated spleen cells following mAb treatment. H, Mean CD19 and IgM MFIs (±SEM) for CFSE<sup>+</sup>CD19<sup>+</sup> and CFSE<sup>+</sup>IgM<sup>+</sup> spleen and lymph node cells.

also significantly reduced in blood 2 days following F(ab’)<sub>2</sub> treatment but returned to normal levels by day 6, in contrast to treatment with intact MB22-11 mAb. Thus, CD22 mAbs alter B cell survival through mechanisms that do not require C3, FcγRs (I, II, III, or IV), or the mAb Fc region.

Discussion

The current study is the first to describe the generation and in vivo assessment of mouse anti-mouse CD22 mAbs that bind to distinct domains of CD22 and elicit ligand-blocking effects (Fig. 1). Using these reagents, this study demonstrates that CD22 ligand binding regulates peripheral B cell survival in vivo, with mAb blockade of CD22 ligand binding leading to significant 2.5- to 4-fold increases in B cell turnover in the blood, spleen, and lymph nodes (Fig. 3A). In contrast, differences in B cell turnover were not observed among nonblocking mAbs, regardless of whether they interacted with CD22 ligand binding domains. Consistent with this result, the survival of adoptively transferred B cells found in bone marrow, blood, spleen, and lymph node was significantly attenuated (73–88%) by mAb blockade of CD22 ligand binding (Fig. 4, A–C), whereas a nonblocking mAb only reduced mature recirculating B cells by 40% and had no effect on B cell survival within spleen or lymph nodes (Fig. 4D). Malignant B cell survival was also significantly reduced (90%) by a CD22 mAb that blocks CD22 ligand binding (Fig. 4E). Inhibiting CD22-ligand interactions through CD22 deletion or mutation of its amino-terminal ligand binding domains is also known to enhance B cell turnover in vivo (24–28). Thereby, CD22 represents an important survival signal for mature follicular B cells, with mAb blockade of ligand engagement resulting in significant and rapid B cell turnover in the periphery.

The MB22-10 and -11 mAbs are likely to bind at or near the CD22 ligand binding site because they blocked trans interactions between CD22 and ligand-bearing splenocytes (Fig. 1, A and B), and their binding required expression of the first two Ig-like domains of CD22 (Fig. 1E). The MB22-11 mAb also completely blocked CD22-Fc fusion protein binding to ligands expressed by T
and B cells, whereas the MB22-10 mAb appeared to partially block binding in this assay system (Fig. 1, C and D). Thereby, the MB22-10 and -11 mAbs may also inhibit CD22-CD22 cis-interactions, which may represent functionally significant signaling units in vivo and regulate CD22 microdomain localization (23, 49, 50). Most important, however, the MB22-10 and MB22-11 mAbs induced significant B cell turnover in vivo and other biological effects beyond those evoked by other CD22 mAbs. CD22 mAbs that bind within the two amino-terminal CD22 ligand binding domains may nonetheless represent a second class of CD22 mAbs that also induce biological effects in vivo. Thus, mAbs that engage the two amino-terminal CD22 ligand binding domains may inhibit physiologically important CD22-ligand interactions in vivo through steric hindrance that are not reflected during in vitro assays, or may enhance CD22 internalization or other functional changes in CD22. Select CD22 ligands that modulate physiologic B cell function in vivo or glycosylation sites on CD22 that mediate cis-interactions have not been identified. Nonetheless, mAbs such as MB22-8 that bind CD22 outside of its ligand binding domains appear to have limited biological effects in vivo. Thus, CD22 mAbs that block CD22-ligand interactions were the most potent inhibitors of CD22-mediated survival signals.

CD22 mAbs that bind epitopes within CD22 ligand binding domains significantly reduced mature bone marrow frequencies (40–63%) and circulating (75–85%) and marginal zone (82–99%) B cell numbers within 3–7 days (Fig. 2). It remains unclear whether CD22 mAbs characterized as “nonblocking” induced these changes by affecting interactions between CD22 and physiologic

**FIGURE 6.** Effects of CD22 mAbs in CD22AA mice and on BCR-induced Ca²⁺ responses and proliferation. A–C, CD22 ligand binding domain mAbs down-modulate CD22 expression in CD22AA mice, but have no effect on CD22AA B cell depletion, turnover, or IgM, CD19, and CD21 expression levels in vivo. CD22AA and wild-type (WT) mice were given 250 μg of MB22-10 or isotype control mAb i.v. (n = 3 mice/group). Splenocytes from wild-type and CD22AA mice were stained and analyzed by flow cytometry 7 days following mAb injection to assess alterations in CD22, IgM, CD19, and CD21 expression levels (A), blood, mature recirculating Ig⁺ B220⁺ bone marrow, and marginal zone B cell numbers (B), and B cell turnover as determined by BrdU staining (C). Values represent mean (±SEM) numbers or percentages of B cells (A and B) or BrdU⁺ B220⁺ B cells (C) within the indicated tissues. *p < 0.05. D, Effect of MB22-10 mAb treatment on BCR-induced Ca²⁺ responses. BCR-induced Ca²⁺ responses in splenocytes from CD22AA, CD22⁻/⁻, or wild-type mice (left panel). BCR-induced Ca²⁺ responses for splenocytes from wild-type (middle panel) or CD22AA (right panel) mice treated for 7 days with MB22-10 or control mAbs in vivo and for splenocytes from untreated mice preincubated for 2 min with MB22-10 mAb (20 μg/ml) in vitro before data collection. Relative Ca²⁺ concentrations were assessed by flow cytometry for B220⁺ gated cells (presented as the fluorescence ratio of 405:525 nm). F(ab’)₂ anti-IgM Ab was added to the cells after 1 min (depicted by the arrow). Ca²⁺ flux responses represent the results obtained in three experiments (left and middle panels) or one experiment (right panel).

E and F, CD22 mAb effects on B cell proliferation in vitro. E, Purified splenic B cells from wild-type mice were CFSE-labeled and incubated in the presence of CD22 or control mAbs, and cultured in medium or with 40 μg/ml F(ab’)₂, anti-IgM, or 0.1–1 μg/ml CD40 mAb. F, Wild-type mice were treated with 250-μg MB22-10 (thick line) or isotype control mAb (thin line) i.v. (n = 2 mice/group). On day 7, splenic B cells were purified, labeled with CFSE, and cultured in medium alone or the presence of 40 μg/ml F(ab’)₂, anti-IgM, 1 μg/ml CD40 mAb, or 2.5 μg/ml LPS. E and F, Fifty thousand events were collected, and cells excluding propidium iodide were analyzed for CFSE fluorescence by flow cytometry.
Figure 7. B cell depletion by CD22 mAbs is independent of C3, FcγRs, and the Fc portion of mAb. A–C, Blood and marginal zone (MZ) B cell numbers (±SEM), and CD22 expression in spleens of C3−/− (A), FcγRIIB−/− (B), and FcγRy−/− (C) mice were assessed 7 days after MB22-10 or isotype-control mAb (250 µg) treatment. In CD22 expression histograms, mice treated with MB22-10 (thick line) or IgG2a isotype control mAb (thin line) are indicated. D, Blood and marginal zone B cell numbers and CD22 expression (day 2 blood and day 6 spleen) in normal mice after one 250 µg of i.v. dose of whole MB22-11, F(ab′)2, MB22-11, or isotype control mAb. Significant differences between means for MB22 or isotype-control mAb-treated mice are indicated; *, p < 0.05.

ligands in vivo or inducing receptor internalization (Fig. 5) thereby rendering B cells unable to interact with ligands due to reduced cell surface CD22 expression. Normally, CD22 is constitutively internalized and subsequently degraded in an acidic intracellular compartment without recycling back to the surface (51). By contrast, mAb binding to CD22 leads to a significant increase in CD22 internalization without increasing its intracellular degradation (51, 52), making CD22 an effective target for the treatment of B cell tumors by immunotoxins (51, 53). Recirculating and marginal zone B cell populations may thereby represent B cell subsets that are exquisitely sensitive to blockade of CD22 ligand binding or mAb-induced reductions in CD22 expression. Indeed, marginal zone B cells were extremely sensitive to treatment with mAbs that bind CD22 ligand binding domains (Fig. 2, G–I). This finding, along with similar reductions in marginal zone B cells observed in CD22Δ1-2, CD22AA, CD22−/−, and ST6Gal-I−/− mice, which lack CD22 ligands, clearly demonstrates that this unique B cell subpopulation requires CD22 ligand-induced signals for survival in vivo (24, 25, 50, 54). Thus, CD22-regulated homeostasis depends on ligands ubiquitously expressed by multiple cell types in vivo (1). Indeed, marginal zone B cells are reported to express higher levels of CD22 in an unmasked form, which would potentially allow for enhanced ligand binding (55). Although a higher proportion of CD22 is similarly reported to be unmasked on B1 cells of the peritoneum (55), inhibition of ligand binding function did not appear to affect this population (data not shown; Ref. 24).

Thus, marginal zone B cells represent a unique B cell subpopulation with enhanced susceptibility to CD22 mutations or mAbs that interfere with CD22 ligand binding.

Although blocking CD22 ligand binding significantly reduced recirculating and marginal zone B cell numbers following mAb treatment, B cell turnover was significantly enhanced in all other tissues (Figs. 2 and 3). CD22AA mice also have significantly higher B cell turnover rates in all compartments, yet normal lymph node and spleen B cell numbers in addition to reduced recirculating and marginal zone B cells numbers (Fig. 6, B and C, and Ref. 24). These differences therefore appear to represent an intrinsic consequence of CD22-ligand interactions within different lymphoid compartments rather than a lack of effective CD22 mAb penetration into tissues. In fact, CD22 mAbs effectively penetrated most tissues within minutes of treatment, with CD22 mAb found at saturating levels on the surface of spleen and lymph node B cells within 1–4 h (K. M. Haas and T. F. Tedder, unpublished observations). In contrast to recirculating blood and marginal zone B cell populations that appear more dependent on CD22-ligand interactions for survival, the spleen and lymph node environments may provide additional survival factors that rescue B cells from depletion in the absence of CD22 ligand binding. For example, CD40-generated signals rescue CD22−/−, CD22Δ1-2, and CD22AA B cell survival following BCR cross-linking in vitro (24, 45). The differences in B cell survival between tissues may also reflect intrinsic differences between the lymphocyte subsets within each tissue as well as their individual turnover rates. Thus, CD22 is one of multiple cell surface receptors that significantly influences B cell survival.

Although CD22 adhesive function was originally envisioned to mediate B cell migration or physical interactions within tissues (12), CD22 engagement of its ligand(s) may primarily promote B cell survival through unknown pathways. In the current study, mature recirculating blood and bone marrow B cell numbers were significantly reduced following CD22 mAb treatment (Figs. 2, A–D, 3B, and 4, A–C). The treatment of mice with either a CD22-Fc fusion protein or polyclonal Abs against CD22 also reduces the mature recirculating IgD+ B cell population in the bone marrow with no change in spleen B cell frequencies (35). Although these results were originally interpreted to suggest that CD22 mediated B cell migration to the bone marrow, B cells from CD22Δ1-2 and CD22AA mice migrate normally to bone marrow and lymphoid tissues (24). Thereby, accelerated B cell turnover due to CD22 blockade is likely to reduce the size of the mature recirculating B cell pool rather than blocking B cell migration to the bone marrow.

The mechanisms through which CD22 mAbs depleted B cells were distinct from those induced by CD20- and CD19-targeted immunotherapies. Effective CD19 and CD20 mAbs deplete B cells in vivo via FcyR-dependent, complement-independent pathways that require mononuclear phagocytes (56–59). CD22 mAb-mediated effects did not require the presence of FcRs (FcγRI, -II, -III, -IV, -VI), -IIIb, and -IVb, and Ref. 24). The treatment of mice with either a CD22-Fc fusion protein or polyclonal Abs against CD22 also reduces the mature recirculating IgD+ B cell population in the bone marrow with no change in spleen B cell frequencies (35). Although these results were originally interpreted to suggest that CD22 mediated B cell migration to the bone marrow, B cells from CD22Δ1-2 and CD22AA mice migrate normally to bone marrow and lymphoid tissues (24). Thereby, accelerated B cell turnover due to CD22 blockade is likely to reduce the size of the mature recirculating B cell pool rather than blocking B cell migration to the bone marrow.

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or -IV) or complement component C3 (Fig. 7, A–C). In fact, intact and F(ab')2 CD22 mAb fragments elicited similar effects, demonstrating that the Fc portion of the mAb was not required for B cell depletion (Fig. 7D). The rapid internalization of CD22 mAbs may be a partial explanation for the lack of dependence on FcRs and complement. However, the MB22-8 mAb did not induce significant CD22 internalization and persisted on the cell surface, but did not significantly affect B cell survival. Nonetheless, FcRs and complement may contribute to the moderate reductions in recirculating B cell numbers caused by the MB22-8 mAb. Although mAb binding to CD22 may also induce B cell FcyRIIb cross-linking, this does not appear to contribute significantly to the results described in this study. Specifically, B cell depletion is similar in FcyRIIb-/- and wild-type mice (Fig. 7B). On CD22AA B cells, CD22 and FcyRIIb may be cross-linked by CD22 mAb, yet this does not affect the phenotype or survival of these cells in vivo or in vitro (Fig. 6, A–D). Moreover, with the exception of inducing CD22 internalization, CD22 ligand blocking mAbs had no measurable effects in CD22AA mice on mature recirculating bone marrow, blood, or marginal zone B cell numbers, B cell turnover, or the expression of other cell surface markers as occurred in wild-type mice (Fig. 6, A–C). These data suggest that CD22 mAbs most likely elicit their effects by interfering with the ability of CD22 to bind its ligands rather than indirect effects.

Wild-type mice treated with CD22 mAbs share similar phenotypes with CD22-/-, CD22AA, and CD22Δ1-2 mice, including significantly reduced numbers of circulating and marginal zone B cells (24). Like B220+/+IgD+ B cells from mice treated with CD22 ligand-blocking mAbs, mature B cells from CD22-/-, CD22Δ1-2, and CD22AA mice express low levels of IgM and CD21, with normal to slightly reduced IgD levels (24, 25, 60). However, CD22 mAb-treated mice (MB22-10) only had slightly elevated levels of MHC class II Ag expression, while B cells from CD22-/-, CD22Δ1-2, and CD22AA mice have significantly elevated MHC class II levels (24, 25, 60). Furthermore, CD22 mAb treatment resulted in a dramatic decrease in CD19 expression by the remaining B cells (Fig. 5, E–H). Whether this phenotypic change directly results from the appearance of a minor B cell subpopulation resistant to CD22 mAb-mediated depletion, CD22 engagement and its effects on B cell signal transduction, or CD22 internalization in vivo is currently unknown. CD22 has been suggested to associate with multiple proteins, including CD19 and IgM (19, 20, 61, 62). Thus, phenotypic changes may be due to internalization of these proteins along with CD22. Interestingly, B cells from CD22AA mice demonstrate no additional alterations in cell surface phenotype following CD22 mAb treatment despite significant CD22 internalization (Fig. 6A). The Ca2+ responses obtained with CD22AA B cells following CD22 mAb treatment in vivo also support this possibility. CD22AA and wild-type mice treated in vivo with MB22-10 mAb generated augmented Ca2+ responses (Fig. 6D), likely due to the loss of cell surface CD22 (Fig. 5, C and D). However, MB22-10 mAb pretreatment in vitro did not alter Ca2+ responses in CD22AA B cells, but did in wild-type B cells. This may reflect the inability of CD22AA molecules to associate with cts-ligands and/or specialized microdomains, such that mAb manipulation had no effect on altering the ability of CD22AA to regulate BCR-mediated Ca2+ responses, while acute mAb engagement of wild-type CD22 augmented Ca2+ responses. Alternatively, phenotypic changes in CD22 mAb-treated mice may be due to the emergence of a minor B cell population that does not depend on CD22 ligand binding for survival. In addition to alterations in cell surface phenotype, B cells from wild-type mice treated with CD22 ligand-blocking mAbs in vitro and B cells from CD22Δ1-2, CD22AA, and CD22-/- mice are hyperproliferative following CD40 stimulation, suggesting that CD22 ligand binding negatively regulates CD40 signaling. Thus, CD22 regulates physiologically relevant ligand-dependent and -independent functions that have distinct effects on B cell biology (24, 45).

The results of this study and the recent characterization of CD22Δ1-2 and CD22AA mice expressing CD22 molecules unable to bind ligands (24) collectively demonstrate that CD22 ligand binding significantly influences B cell turnover and is required for normal B cell survival in the periphery. Similarly, mAb blockade of CD22 ligand binding inhibits human B cell survival in vitro, and either induces or inhibits B cell signal transduction (36–38, 63). These anti-human CD22 mAbs are also tumoridal in xenotransplantation models of human lymphomas (38). In the current studies, mAb blockade of mouse CD22 ligand binding also significantly reduced the survival of A20 tumor cells in vivo (Fig. 4E), suggesting the ability of CD22 to regulate both normal and malignant B cell survival. This raises the possibility that mAb antagonists that interfere with CD22 ligand binding may demonstrate therapeutic effectiveness for both B cell malignancies and some autoimmune diseases. Moreover, the current studies clearly demonstrate that CD22 mAbs have variable effects on B cells in vivo depending on the region of CD22 bound. Thereby, mAbs that block human CD22 ligand binding may have effects on B cells that are not induced by mAbs that target nonligand binding domains of CD22. Indeed, an anti-human CD22 ligand-blocking mAb, HB22-7, exhibits increased cytotoxic effects on human CD22+ tumor cells in mice compared with nonblocking mAbs (37, 38). Interestingly, the therapeutic anti-human CD22 mAb, LL2 (epratuzumab), binds to a region outside of the two amino-terminal ligand binding domains of CD22 (64). Thus, a further understanding of the factors that influence CD22 ligand interactions may lead to novel therapies that inhibit the survival of pathogenic B cells.

Disclosures
T. F. Tedder is a consultant and equity holder in Angelica Therapeutics Inc. and also a consultant for MedImmune Inc.

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