Differentially Regulated Expression and Function of CD22 in Activated B-1 and B-2 Lymphocytes[1]

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CD22 is a B cell-restricted transmembrane protein that apparently controls signal transduction thresholds initiated through the B cell Ag receptor (BCR) in response to Ag. However, it is still poorly understood how the expression of CD22 is regulated in B cells after their activation. Here we show that the expression levels of CD22 in conventional B-2 cells are markedly down-regulated after cross-linking of BCR with anti-IgM mAb but are up-regulated after stimulation with LPS, anti-CD40 mAb, or IL-4. In contrast, treatment with anti-IgM mAb barely modulated the expression levels of CD22 in $CD5^+$ B-1 cells, consistent with a weak Ca^{2+} response in anti-IgM-treated $CD5^+$ B-1 cells. Moreover, in CD22-deficient mice, anti-IgM treatment did not trigger enhanced Ca^{2+} influx in $CD5^+$ B-1 cells, unlike CD22-deficient splenic B-2 cells, suggesting a relatively limited role of CD22 in BCR signaling in B-1 cells. In contrast, CD22 levels were markedly down-regulated on wild-type B-1 cells in response to LPS or unmethylated CpG-containing oligodeoxynucleotides. These data indicate that the expression and function of CD22 are differentially regulated in B-1 and conventional B-2 cells, which are apparently implicated in innate and adaptive immunity, respectively. ***The Journal of Immunology,*** **2002, 168: 6078–6083.**

CD22, a B cell-specific member of the Ig superfamily with seven Ig-like domains, functions as a coreceptor for the B cell Ag receptor (BCR)[3] (1, 2) and is also an adhesion receptor recognizing α2,6-linked sialic acid-bearing glycans on target cells (3–5). First appearing on the surface of pre-B cells, CD22 is fully expressed by mature IgM^+IgD^+ B cells and finally lost on terminally differentiated plasma cells (6, 7). Upon BCR cross-linking, the cytoplasmic domain of CD22 is rapidly tyrosine-phosphorylated, resulting in recruitment of a number of signaling molecules, including tyrosine kinases (Lyn and Syk), phospholipase C-γ1, and phosphatidylinositol 3-kinase (8, 9). However, tyrosine-phosphorylated CD22 recruits and activates SH2 domain-containing protein tyrosine phosphatase (SHP-1) (10), which negatively regulates BCR signaling (11), and CD22-deficient splenic B cells exhibit a greatly enhanced and prolonged Ca^{2+} signal after BCR stimulation (6, 12–14). These findings suggest that CD22 functions primarily as a negative regulator of BCR signaling by controlling signal transduction threshold initiated through BCR in B cells in response to Ag (1, 2).

B-1 cells differ from conventional peripheral B cells (B-2) by their anatomical location, Ag specificity, surface markers, and their potential for self-renewal (15). B-1 cells are the predominant B cell population in the peritoneal cavity, but rare in spleen and lymph nodes of adult mice (16). Abs secreted by B-1 cells are primarily polyreactive IgM of low affinity and cross-react with a variety of self Ags (17–20). Ab production by B-1 cells was induced by multivalent T cell-independent bacterial polysaccharide Ags (18, 21, 22), whereas B-2 cells recognize a wide variety of Ags with high affinity. B-1 cells are also distinguished from B-2 cells by their unique surface markers such as CD5, Mac-1, and lower B220 expression. It has recently been shown that CD5 plays the role of a negative regulator in BCR-mediated proliferation of B-1 cells (23), likely by recruiting SHP-1 (24), but it is still not well defined how CD22 is implicated in the activation of B-1 cells upon their stimulation.

In view of the primary role of CD22 as a negative regulator of BCR signaling, its regulated expression is likely to determine signal transduction thresholds initiated through BCR, and thus in turn B cell responses to foreign Ags and self Ags. Although LPS or CD40 ligation in the presence of IL-4 up-regulates the expression of CD22 (7, 25), the control of CD22 expression in response to BCR cross-linking is still poorly understood. Therefore, in the present study, we have conducted a systematic analysis of CD22 expression in both B-1 and B-2 cells in response to different activation stimuli. In addition, we compared the influence of CD22 on Ca^{2+} signaling between these two subsets of B cells.

Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). $CD22^{-/-}$ mice with a pure B6 background were developed as described previously (6, 7). B6 mice deficient in FcγRII (26) or bearing the X-linked immunodeficiency (*Xid*) mutation (deficient in the Btk kinase) (27) were respectively provided by Dr. J. Ravetch (Rockefeller University, New York, NY) and Dr. A. Hugins (Geneva, Switzerland).

Cell culture

Spleen or peritoneal cavity cells ($1–2 \times 10^6$) from a pool of three to five mice at 2–4 mo of age were incubated in 1 ml of DMEM containing 10% FCS in Falcon 24-well plates (BD Labware, Franklin Lakes, NJ) in the presence of LPS, b7-6 rat anti-IgM (28), Bet-2 rat anti-IgM (29), $F(ab')_2$

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[3] Abbreviations used in this paper: BCR, B cell Ag receptor; B6, C57BL/6; *Xid*, X-linked immunodeficiency; CpG-ODN, unmethylated CpG-containing oligodeoxynucleotides; SHP-1, SH2 domain-containing protein tyrosine phosphatase.

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goat anti-IgM (Jackson ImmunoResearch Laboratories, Hamburg, Germany), FGK45 rat anti-CD40 mAb (a gift from Dr. J. Andersson, Basel Institute for Immunology, Basel, Switzerland), different cytokines or unmethylated CpG-containing oligodeoxynucleotides (CpG-ODN; 5′-TCCATGA CGTTCCTGACGTT-3′; Microsynth, Balgach, Switzerland) for 24, 48, or 72 h. Optimal concentrations of each stimulator were predetermined by the measurement of proliferative responses or up-regulation of MHC class II molecules on B cells. Then, the expression of CD22, MHC class II, and CD86 molecules on B cells was determined by flow cytometric analysis. Polyclonal rat IgG purified from pooled rat serum by protein G column chromatography was used as control. For some experiments, B cells from peritoneal lavage cells were purified by complement-mediated T cell lysis with anti-CD4 and anti-CD8 mAb and the removal of adherent macrophages by an overnight incubation on tissue culture plastic wells. After this procedure, the remaining cells were ~95% B lineage cells, as determined by flow cytometric analysis. These cells were then stimulated with either b7-6 anti-IgM mAb (25 μg/ml) or F(ab′)$_2$ goat anti-IgM (10 μg/ml).

Flow cytometric analysis

Flow cytometry was performed using two-color staining of lymphocytes and analyzed with a FACSCalibur (BD Biosciences). The following Abs and reagents were used: FITC-labeled NIM-R6 rat anti-CD22 (30); FITC- or PE-conjugated RA3-6B2 rat anti-B220 (BD PharMingen, San Diego, CA); FITC- or biotin-labeled Y-3P rat anti-I-A (BD PharMingen); FITC-labeled anti-CD86 (BD PharMingen); PE- or biotin-labeled 53-7.3 rat anti-CD5 (BD PharMingen); PE-labeled goat anti-IgM (BD PharMingen); and PE-labeled streptavidin (BD PharMingen).

Northern blot analysis

Total RNA was prepared from spleen cells by RNeasy Mini kit (Qiagen, Basel, Switzerland). RNA (5 μg) was electrophoresed on a 1% agarose gel, transferred to nylon membrane, and hybridized with a ^{32}P-labeled murine CD22 cDNA (30). The CD22 mRNA levels were quantified by determining the ratios of the intensities of CD22 mRNA bands and methylene blue-stained 18S rRNA bands by densitometric analysis.

Measurement of intracellular Ca^{2+} mobilization

Spleen or peritoneal cavity cells (10^7) from a pool of three to five CD22$^{-/-}$ and wild-type B6 mice were loaded with 4.5 μM Indo-1 (Molecular Probes, Eugene, OR) and 0.003% pluronic F-127 in RPMI (pH 7.4) with 1% FCS for 45 min at 37°C. After Indo-1 loading, cells were stained on ice with FITC-labeled anti-B220 and PE-labeled anti-CD5 mAb. Cells were washed and IgM on the B cell surface was cross-linked at 37°C with 30 μg/ml b7-6 anti-IgM mAb. Increases of intracellular Ca^{2+} in splenic B-2 cells (gated B220$^+$CD5$^-$) or peritoneal B-1 cells (gated B220$^+$CD5$^+$) were recorded in real time for 8 min with the use of a FACSVantage (BD Biosciences). The anti-B220 or anti-CD5 pretreatment on ice had no effect on Ca^{2+} flux, as was checked by comparison to unstained B cells.

Results

Increased CD22 expression on splenic B cells after stimulation with LPS, anti-CD40 mAb, or IL-4 but decreased expression on B cells after activation with anti-IgM mAb

It has been previously shown that CD22 levels were increased after stimulation of splenic B cells with LPS or CD40 ligand in the presence of IL-4 (7, 25). To confirm this observation, splenic B cells from 2-mo-old B6 mice were stimulated with either LPS or anti-CD40 mAb at optimal concentrations of each stimulator (25 μg/ml). Intensities of surface CD22 staining on B cells, as assessed by flow cytometry using NIM-R6 anti-CD22 mAb, were significantly increased on B220$^+$ B cells at 24 h and remained at high levels after 48 and 72 h of stimulation with either LPS or rat anti-CD40 mAb (Fig. 1 and 2), but not with polyclonal rat IgG (data not shown). CD22 expression levels were also up-regulated after the stimulation with IL-4 (500 U/ml) but were unchanged after the treatment with IFN-γ, IL-1, IL-6, or TNF at any dose tested (data not shown).

We next examined whether CD22 expression levels can be similarly up-regulated after the activation of B cells by cross-linking of BCR with anti-IgM mAb. When different concentrations (1, 5, or 25 μg/ml) of b7-6 anti-IgM mAb were tested, CD22 surface

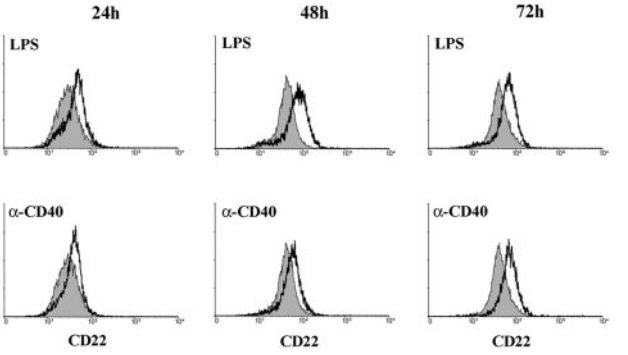

FIGURE 1. Increased surface expression of CD22 on B-2 cells after stimulation with LPS or anti- (α-)CD40 mAb. Spleen cells from 2-mo-old B6 mice were incubated with 25 μg/ml LPS or anti-CD40 mAb for 24, 48, and 72 h, and the expression levels of CD22 were assessed by incubation with FITC-labeled rat anti-CD22 mAb (NIM-R6) and PE-conjugated anti-B220 mAb. Fluorescence intensities of CD22 on stimulated (dark lines) and unstimulated (shaded) B220$^+$ B cells are shown.

expression was substantially diminished at 48 h and further down-regulated at 72 h in a dose-dependent manner (Fig. 3*A*). At 72 h, mean fluorescence intensity of CD22 in B cells stimulated with 25 μg/ml anti-IgM mAb became ~40% of that of control unstimulated B cells (Fig. 2). The down-regulated expression of CD22 in B cells stimulated with anti-IgM mAb contrasted markedly with an up-regulated expression of I-A molecules on the same B cells (Fig. 3*B*). Essentially identical results were obtained with another anti-IgM mAb, Bet-2, or F(ab′)$_2$ goat anti-IgM polyclonal Abs (data not shown). An inhibitory role of FcγRII for the expression of CD22 after BCR cross-linking by anti-IgM mAb, possibly coengaging FcγRII, was excluded, because CD22 on FcγRII-deficient B cells was similarly down-regulated after the activation with anti-IgM mAb (Fig. 2). To determine the possible role of BCR-mediated CD22 internalization after anti-IgM treatment, the extent of CD22 down-modulation was assessed on spleen B cells bearing the *Xid* mutation, which are known to be defective in certain BCR-triggered events, such as proliferation (31). *Xid* B cells internalized BCR as efficiently as wild-type B cells, as judged by decreased surface staining with polyclonal goat anti-IgM conjugates 1, 3, and 6 h after the incubation with anti-IgM mAb (data not shown). In

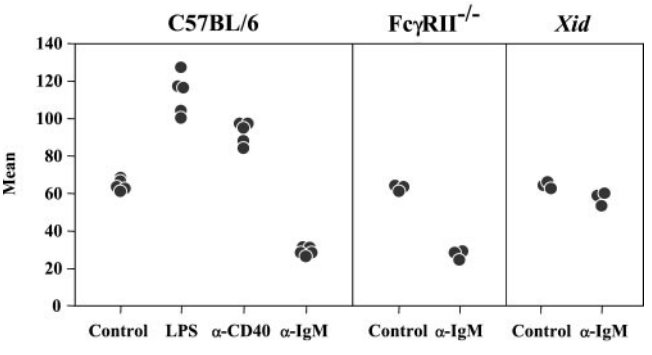

FIGURE 2. Modulation of CD22 surface expression on B-2 cells after stimulation with LPS, anti- (α-)CD40, or anti-IgM mAb. Spleen cells from 2-mo-old B6, FcγRII-deficient B6 or *Xid* B6 mice were incubated with 25 μg/ml LPS, anti-CD40, or b7-6 anti-IgM mAb for 72 h, and the expression levels of CD22 were assessed by incubation with FITC-labeled rat anti-CD22 mAb (NIM-R6) and PE-conjugated anti-B220 mAb. Results from three to five individual mice, expressed as mean fluorescence intensities of CD22 on B220$^+$ B cells, are shown. Background fluorescence intensity on B220$^-$ cells was <3.0.

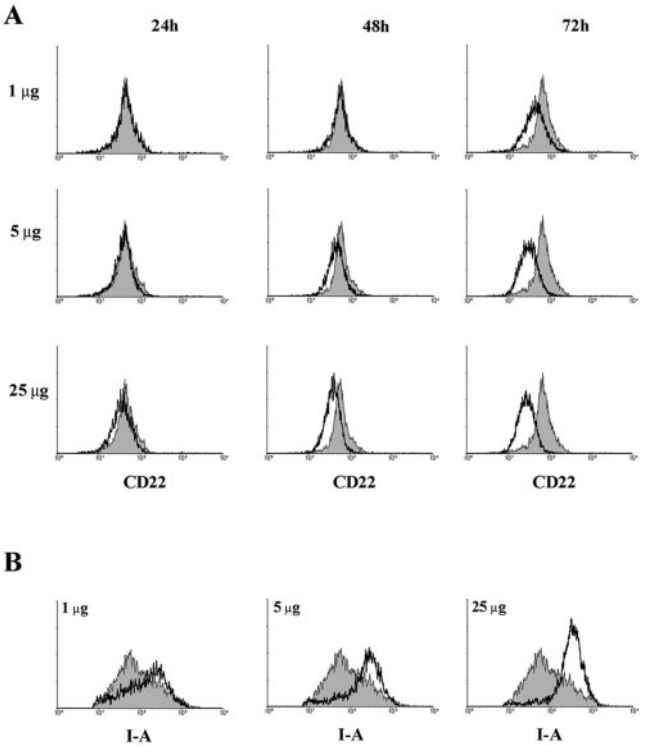

FIGURE 3. Reduced surface expression of CD22 and enhanced expression of I-A on B-2 cells after stimulation with anti-IgM mAb. Spleen cells from 2-mo-old B6 mice were incubated with 1, 5, or 25 μg/ml of b7-6 anti-IgM mAb for 24, 48 and 72 h, and the expression levels of CD22 were assessed by incubation with FITC-labeled rat anti-CD22 (NIM-R6) or FITC-labeled rat anti-I-A (Y-3P) and PE-conjugated anti-B220 mAb. *A,* Fluorescence intensities of CD22 on stimulated (dark lines) and unstimulated (shaded) $B220^+$ B cells 24, 48, and 72 h after the incubation with the indicated concentrations of anti-IgM mAb. *B,* Fluorescence intensities of I-A on stimulated (dark lines) and unstimulated (shaded) $B220^+$ B cells after 24 h of incubation with anti-IgM mAb.

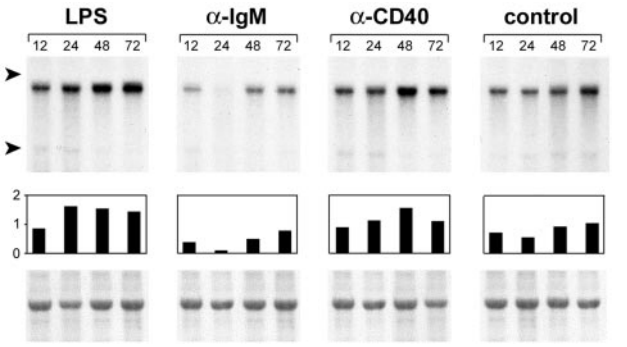

FIGURE 4. Northern blot analysis of CD22 mRNA from B6 spleen cells stimulated with LPS, anti- (α-)CD40, or b7-6 anti-IgM mAb. At indicated time points, cultures were harvested, and total RNA was isolated and analyzed by Northern blotting. Positions of 18S and 28S rRNA are indicated. The CD22 mRNA levels were quantified by determining the ratios of the intensities of CD22 mRNA bands and methylene blue-stained 18S bands by densitometric analysis.

contrast, the level of CD22 was only poorly down-regulated in anti-IgM-treated *Xid* B cells, as compared with wild-type B cells (Fig. 2).

To determine whether the increased or decreased levels of CD22 on differently activated B cells were paralleled by the modulation of CD22 mRNA abundance, the levels of mRNA coding for CD22 were assessed by Northern blot analysis on spleen cells after the stimulation with either LPS, anti-CD40 or anti-IgM mAb. The treatment with either LPS or anti-CD40 mAb led to an increase of CD22 mRNA levels at 24 and 48 h, whereas anti-IgM mAb treatment markedly down-regulated the level of CD22 mRNA at 24 h, as compared with control cultures (Fig. 4).

Lack of CD22 modulation on B-1 cells treated with anti-IgM mAb and failure to enhance Ca^{2+} response in CD22-deficient B-1 cells treated with anti-IgM mAb

Because it has been described that $CD5^+$ B-1 cells express CD22 at levels comparable with those of conventional B-2 cells (7, 32), we determined whether CD22 expression can be modulated on B-1 cells following the stimulation with anti-IgM mAb in a way similar to that of B-2 cells. When the expression levels of CD22 on peritoneal lavage cells from 3- to 4-mo-old B6 mice were analyzed after 48 h of stimulation with anti-IgM mAb, no down-regulation of CD22 expression was observed in $CD5^+$ B-1 cells (Fig. 5*A*). In contrast, the stimulation with anti-IgM mAb substantially reduced the levels of CD22 on the $CD5^-$ population of peritoneal B cells. To determine whether other BCR-mediated signaling events are blocked in B-1 cells, additional studies were conducted to evaluate the expression of I-A and costimulatory CD86 molecules. As shown in Fig. 5*B,* the treatment with anti-IgM mAb led to an up-regulated expression of I-A and CD86 molecules in the $CD5^-$ population, but not in $CD5^+$ B-1 cells (Fig. 5*B*). Notably, essentially identical results were obtained with $F(ab')_2$ goat anti-IgM polyclonal Abs (data not shown).

The lack of down-regulation of CD22 within the $CD5^+$ B-1 population after anti-IgM stimulation suggested a different role of CD22, as compared with splenic B-2 cells. To address whether the BCR signal on B-1 cells is indeed negatively regulated by CD22, we stimulated peritoneal B-1 cells and splenic B-2 cells from CD22-deficient and wild-type B6 mice with ant-IgM mAb and measured intracellular Ca^{2+} mobilization. Peritoneal wild-type $CD5^+$ B-1 cells showed a weaker Ca^{2+} response than splenic B-2 cells (Fig. 6), consistent with the lack of down-modulation of CD22 and the lack of up-regulation of I-A and CD86 in this population. Significantly, the $CD5^+$ B-1 cells from CD22-deficient mice only gave a weak Ca^{2+} response, not higher than that seen in the B-1 cells of wild-type mice. In contrast, a strongly enhanced Ca^{2+} response was observed in CD22-deficient B cells from the spleen, as expected (6, 7).

Marked down-regulation of CD22 expression on peritoneal B-1 cells after stimulation with LPS or CpG-ODN

Because $CD5^+$ B-1 cells failed to modulate CD22 upon BCR cross-linking by anti-IgM mAb, we determined whether LPS stimulation could up-regulate CD22 expression, as observed in splenic B cells. When peritoneal lavage cells of B6 mice were stimulated with LPS, we observed a marked reduction of CD22 expression in the majority of $CD5^+$ B-1 cells and no up-regulation in the rest of the B-1 cells (Fig. 7). This was in contrasted with an increased expression of CD22 on the $CD5^-$ population of peritoneal B cells similar to that observed with splenic B cells.

A marked down-regulation of CD22 upon LPS stimulation could be a unique feature of B-1 cells in response to nonspecific B cell activators of microbial origin to promote immune responses against bacterial Ags. This is in agreement with the fact that B-1 cells are involved in T-independent Ab responses against bacterial polysaccharide Ags (22, 33). Therefore, we also tested the effect of an immunostimulatory oligodeoxynucleotide containing an unmethylated CpG motif present in bacterial, but not vertebrate DNA

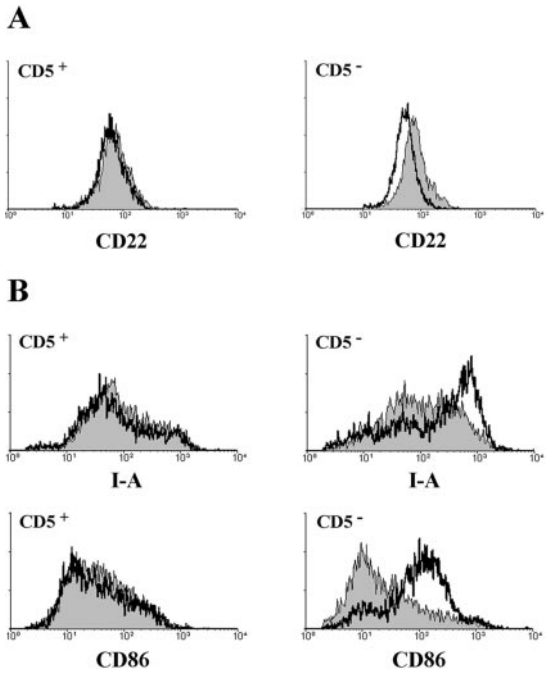

FIGURE 5. Lack of modulation of CD22, I-A, and CD86 on $CD5^+$ B-1 cells after stimulation with anti-IgM mAb. *A*, Peritoneal lavage cells from 3- to 4 mo-old B6 mice were incubated with 25 μg/ml b7-6 anti-IgM mAb for 48 h, and the expression levels of CD22 were assessed by FITC-labeled rat anti-CD22 mAb (NIM-R6) and biotinylated anti-CD5 mAb. Fluorescence intensities of CD22 on stimulated (dark lines) and unstimulated (shaded) $CD5^+$ and $CD5^-$ B cells are shown. Representative results of three separate experiments are shown. *B*, Peritoneal B cells purified from 3- to 4-mo-old B6 mice were incubated with 25 μg/ml b7-6 anti-IgM mAb for 24 h, and the expression levels of I-A and CD86 were assessed by using biotinylated anti-CD5 mAb and FITC-labeled anti-I-A or anti-CD86 mAb. Fluorescence intensities of I-A and CD86 on stimulated (dark lines) and unstimulated (shaded) $CD5^+$ and $CD5^-$ B cells, representative of two separate experiments, are shown. The analysis of CD22 in these experiments using purified B cells also confirmed the lack of CD22 modulation on $CD5^+$ B-1 cells treated with anti-IgM mAb (data not shown).

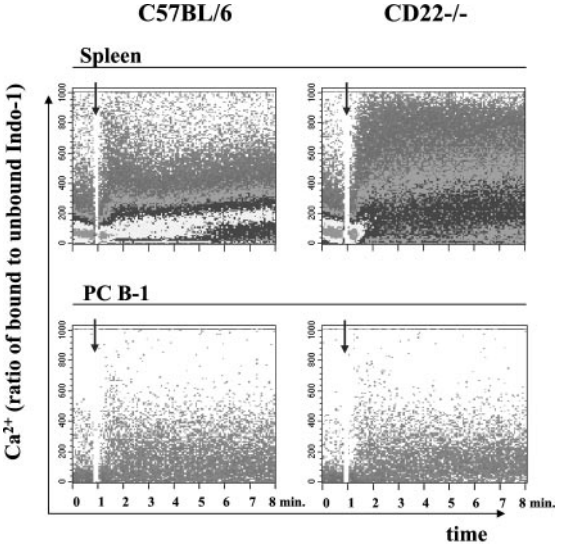

FIGURE 6. Lack of enhancement of Ca^{2+} response in CD22-deficient B-1 cells treated with anti-IgM mAb. Cells from spleen or peritoneal cavity were stimulated with 30 μg/ml b7-6 anti-IgM mAb for 8 min. Intracellular Ca^{2+} concentrations are represented as the ratio of bound to unbound Indo-1. Color density blots indicate the percentage of cells with certain Ca^{2+} concentrations at a given time point. The shown diagrams are for $B220^+$ cells in the spleen and for $CD5^+B220^+$ B-1 cells in the peritoneal cavity (PC B-1). Pretreatment with anti-CD5 and anti-B220 Abs on ice had no effect on Ca^{2+}, as controlled with unstained cells. Arrows, Addition of anti-IgM mAb. $CD5^+$ B-1 cells of CD22-deficient mice showed no enhanced Ca^{2+} flux after anti-IgM stimulation, in contrast to the enhanced Ca^{2+} response in CD22 -deficient splenic B-2 cells.

(34) on the expression of CD22 on peritoneal B-1 cells and conventional splenic B-2 cells. As for LPS stimulation, CD22 levels were markedly reduced on nearly all $CD5^+$ B-1 cells after activation with CpG-ODN (Fig. 7). In addition, we observed a significant down-regulation of CD5 on B-1 cells after the activation with CpG-ODN, but not LPS (data not shown). In contrast, CpG-ODN stimulation did not induce substantial changes in CD22 expression levels on $CD5^-$ peritoneal and splenic B cells, although the levels of CD22 expression became somehow more heterogeneous in splenic B cells (Fig. 7).

Discussion

In the present study, we have assessed the modulation of CD22 in activated B cells in relation to the B-1 and B-2 phenotypes. Our results revealed several important points. First, the expression levels of CD22 in conventional splenic B-2 cells were markedly down-regulated after activation via the BCR by anti-IgM mAb, in contrast to an up-regulated CD22 expression after activation with LPS, anti-CD40 mAb, or IL-4. Second, CD22 expression was differently regulated in $CD5^+$ B-1 cells after the activation with LPS or anti-IgM mAb; their CD22 levels were hardly modulated after stimulation with anti-IgM mAb but markedly down-regulated in response to LPS. Strikingly, a similar down-regulation of CD22 was also observed in $CD5^+$ B-1 cells after stimulation with immunostimulatory CpG-ODN. Finally, anti-IgM treatment did not trigger enhanced Ca^{2+} influx in $CD5^+$ B-1 cells from CD22-deficient mice, in contrast to a strongly increased Ca^{2+} response of CD22-deficient B-2 cells from the spleen compared with wild-type B-2 cells. Our results thus demonstrate that the expression and function of CD22 is differentially regulated in B-1 and conventional B-2 cells.

The first significant observation in the present study is the marked down-regulation of CD22 in conventional B-2 cells after cross-linking of BCR with anti-IgM mAb. This is somehow surprising because other B cell stimulators, such as LPS, anti-CD40 mAb, or IL-4, uniformly up-regulated the expression levels of CD22 in these cells. This difference apparently resulted from a differential regulation of the expression levels of CD22 mRNA after BCR-mediated and -independent stimulation of B-2 cells. A rapid loss of CD22 mRNA, peaking at 24 h after anti-IgM treatment, likely accounts for the down-modulation of CD22 expression on B-2 cells. The down-regulation of CD22 in anti-IgM mAb-treated B-2 cells could in part be related to the cointernalization of CD22 with BCR during culture, because endocytosed CD22 appears to be rapidly degraded (35). However, it should be stressed that only a small fraction ($<5\%$) of total CD22 apparently associates with BCR (36, 37). Moreover, we observed limited CD22 down-modulation on anti-IgM-treated *Xid* B cells defective in certain BCR-triggered signaling, but not BCR internalization, which strongly argues against the idea that BCR-mediated CD22 cointernalization plays a major role in anti-IgM-induced CD22 down-regulation on B-2 cells.

The observed specific and selective down-regulation of CD22 expression in B-2 cells after cross-linking of the BCR may be relevant for the development and regulation of immune responses to foreign Ags and self Ags. It is now well established that CD22

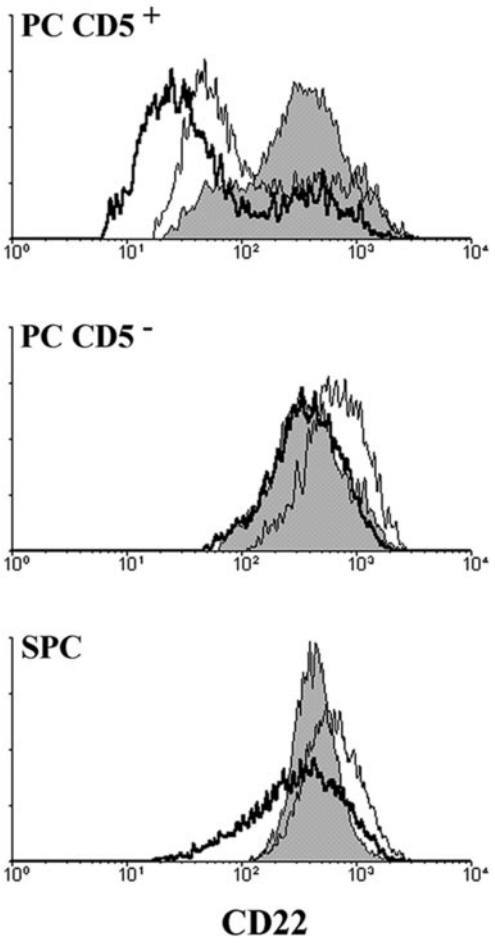

FIGURE 7. Differential regulation of CD22 on $CD5^+$ B-1 and $CD5^-$ B cells after stimulation with LPS or immunostimulatory CpG-ODN. Peritoneal lavage and spleen cells from 3- to 4-mo-old B6 mice were incubated with 25 μg/ml LPS or 3 μg/ml CpG-ODN for 48 h, and the expression levels of CD22 were assessed by FITC-labeled rat anti-CD22 mAb (NIM-R6), followed by biotinylated anti-CD5 mAb and PE-streptavidin. Fluorescence intensities of CD22 on CpG-ODN-stimulated (thick lines), LPS-stimulated (thin lines) and unstimulated (shaded) $CD5^+$ and $CD5^-$ B cells in the peritoneal cavity (PC) and conventional B-2 cells from spleen (SPC), representative of three separate experiments, are shown.

becomes rapidly tyrosine phosphorylated on its cytoplasmic tail upon cross-linking of BCR and recruits SHP-1, thereby negatively regulating BCR signaling (1, 2). Thus, it is conceivable that the down-regulation of CD22 in B-2 cells after BCR cross-linking could result in a diminished number of CD22 molecules associated with recycled or newly generated BCR. Consequently, BCR-mediated signaling and hence B cell responses to Ag could be promoted. In contrast, the up-regulated expression of CD22 in B cells activated by BCR-independent stimulators, such as LPS, anti-CD40 mAb or IL-4, may help prevent unwanted activation of B cells bearing BCR with low affinity binding or with autoreactivity, emerging as a result of somatic hypermutations during immune responses. Thus, dysregulated expression of CD22 could lead to excessive activation of B cells and autoantibody production, as in the case of mice deficient in CD22 (12, 25, 38). Genome wide mapping analysis for lupus susceptibility loci in autoimmune-prone New Zealand White (NZW) mice revealed that an interval containing the *Cd22* gene on chromosome 7 is linked with autoantibody production and lupus-like glomerulonephritis (39–41). More recently, after the activation of B cells with LPS in the presence of IL-4, CD22 expression was less up-regulated in NZW mice bearing the $Cd22^a$ allele, as compared with B6 mice bearing the $Cd22^b$ allele (25). This is apparently related to the synthesis of abnormally processed CD22 mRNA, in addition to the wild-type transcripts, as a result of a short interspersed nucleotide element insertion in the second intron of the $Cd22^a$ gene. Thus, defective up-regulation of CD22 on potentially autoreactive B cells stimulated nonspecifically during immune responses may favor the production of autoantibodies in lupus-prone mice bearing the $Cd22^a$ allele, such as (New Zealand Black $\times$ NZW)F_1 hybrid mice.

In contrast to conventional B-2 cells, the expression of CD22 is controlled differently in $CD5^+$ B-1 cells, which are involved in the production of natural and pathogenic autoantibodies (18–20, 42) and in the T cell-independent Ab responses against bacterial polysaccharide Ags (22, 33). The stimulation with anti-IgM mAb barely modulated CD22 expression levels in B-1 cells. This may in part be related to the expression of CD5 on these cells, which negatively controls BCR-mediated signaling (23), likely by recruitment of SHP-1 (24, 43). This is consistent with the finding that the anti-IgM treatment led to weak Ca^{2+} mobilization and failed to up-regulate the expression level of I-A and CD86 in B-1 cells. In addition, we observed that the early BCR signaling appears to be poorly regulated by CD22 in B-1 cells, because $CD5^+$ B-1 cells lacking CD22 failed to exhibit enhanced Ca^{2+} flux responses, unlike conventional splenic B-2 cells. This markedly contrasted with the finding that B-1 cells of CD5-deficient mice exhibited a more sustained Ca^{2+} flux and were able to undergo proliferation after IgM stimulation, compared with control B-1 cells (23). These data suggest that CD5 functions as the major negative regulator of BCR signaling in B-1 cells.

Furthermore, we observed an expansion of the majority of $CD5^+$ B-1 cells with markedly down-regulated CD22 expression after the stimulation with LPS. Interestingly, immunostimulatory CpG-ODN, the sequence of which is specifically present in bacterial DNA, also markedly reduced the expression levels of CD22 on nearly the entire population of $CD5^+$ B-1 cells. Because we have not performed the experiments with purified B-1 cells, it remains to be determined whether the down-regulation of CD22 was due to a direct effect of LPS or CpG-ODN on B-1 cells or secondary to cytokines secreted by macrophages activated with LPS or CpG-ODN. Although the negative role of CD22 for BCR signaling in $CD5^+$ B-1 cells is apparently less prominent than in conventional B-2 cells, one cannot exclude the possibility that the observed marked down-regulation of CD22 by LPS or CpG-ODN could be a mechanism to reduce BCR signaling thresholds in B-1 cells expressing CD5. In this regard, it should be stressed that the level of CD5 was also significantly down-regulated in B-1 cells treated with CpG-ODN, thereby further promoting the activation of B-1 cells specific for bacterial Ags.

In conclusion, we have demonstrated that the expression of CD22 is differentially regulated after BCR-mediated and -independent activation in B-1 and B-2 cells, which are apparently implicated in innate and adaptive immunity, respectively (17, 44). In addition, our data suggest that CD5 is a more potent negative regulator of BCR signaling than CD22 in B-1 cells, contrary to conventional B-2 cells. In view of the potential role of CD22 in the development of lupus-like autoimmune diseases (12, 25, 38, 41), further assessment of the regulation of CD22 expression in mice bearing different allelic forms of CD22 should help understand a possible role of CD22 polymorphism in the development of autoimmune and other diseases in which B cell function is dysregulated.

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