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

Frédéric Lajaunias, Lars Nitschke, Thomas Moll, Eduardo Martinez-Soria, Isabelle Semac, Yves Chicheportiche, R. Michael E. Parkhouse and Shozo Izui


http://www.jimmunol.org/content/168/12/6078

**References**

This article cites 44 articles, 24 of which you can access for free at: http://www.jimmunol.org/content/168/12/6078.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Differentially Regulated Expression and Function of CD22 in Activated B-1 and B-2 Lymphocytes

Frédéric Lajaunias,* Lars Nitschke, † Thomas Moll,* Eduardo Martinez-Soria,* Isabelle Semac,* Yves Chicheportiche,‡ R. Michael E. Parkhouse,§ and Shozo Izui‡

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 Ca²⁺ response in anti-IgM-treated CD5⁺ B-1 cells. Moreover, in CD22-deficient mice, anti-IgM treatment did not trigger enhanced Ca²⁺ 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)⁵ (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 Ca²⁺ 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 Ca²⁺ 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 FcγRIII (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 × 10⁶) 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), P1a(1)₂.
Flow cytometric analysis
Flow cytometry was performed using two-color staining of lymphocytes and analyzed with a FACS Calibur (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 32P-labeled cDNA probe of CD22 (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 Ca2+ mobilization
Spleen or peritoneal cavity cells (107) 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) at 37°C for 15 min. After Indo-1 loading, cells were stained on ice with FITC-labeled anti-B220 and PE-labeled anti-CD5 mAb. Cells were washed and resuspended in RPMI 1640 medium containing 10% FCS and 10 μM of Indo-1 (Molecular Probes). The flow cytometric analysis was performed using the FACSVantage system (BD Biosciences). The CD22-/- or anti-CD5 pretreatment on ice had no effect on Ca2+ flux, as was checked by comparison to unstimulated 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 stimulus (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. 1A 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 expression was substantially diminished at 48 h and further down-regulated at 72 h in a dose-dependent manner (Fig. 3A). 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. 3B). 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 co-engaging 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
of CD22 expression was observed in CD5− B-1 cells (Fig. 5) when compared with CD5− B-2 cells. In 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 Ca2+ 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. 5A). 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. 5B, 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. 5B). 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 anti-IgM mAb and measured intracellular Ca2+ mobilization. Peritoneal wild-type CD5− B-1 cells showed a weaker Ca2+ 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 Ca2+ response, not higher than that seen in the B-1 cells of wild-type mice. In contrast, a strongly enhanced Ca2+ 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.
unstimulated (shaded) CD5 Fluorescence intensities of I-A and CD86 on stimulated (dark lines) and biotinylated anti-CD5 mAb and FITC-labeled anti-I-A or anti-CD86 mAb. for 24 h, and the expression levels of I-A and CD86 were assessed by using Peritoneal lavage cells from 3- to 4-mo-old B6 mice were incubated with 25 μg/ml b7-6 anti-IgM mAb (data not shown). In contrast, CpG-ODN up-regulated CD5 in B-2 cells. The down-regulation of CD22 in anti-IgM mAb-treated B cells could in part be related to the cointernalization 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-regulation 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
peritoneal lavage and spleen cells from 3- to 4-mo-old B6 mice were incubated with bacterial DNA, also markedly reduced the expression levels of CD22 after the stimulation with LPS. Interestingly, immunostimulatory CpG-ODN, the sequence of which is specified in bacterial DNA, also markedly reduced the expression levels of CD22 in 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.

Acknowledgments
We thank Guy Brighouse, Giuseppe Celetta, Sonja Rotzoll, and Astrid Heiter for their excellent technical help.
References
6. Nitschke, L., R. Carsetti, B. Ocker, G. Kohler, and M. C. Lammers. 1997. CD22 is a negative regulator of B cell receptor signaling. Curr. Biol. 7:133.