Engagement of the Adhesion Receptor CD22 Triggers a Potent Stimulatory Signal for B Cells and Blocking CD22/CD22L Interactions Impairs T-Cell Proliferation

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The B-lymphocyte-restricted adhesion protein CD22 mediates sialic acid-dependent cell-cell interactions. Engagement of CD22 on B lymphocytes with a CD22 monoclonal antibody (MoAb) HB22.7 that blocks the binding of CD22 to its ligand(s) directly stimulated B-cell proliferation. In addition, the HB22.7 MoAb costimulated B-cell proliferation with either anti-IgM, interleukin-2 (IL-2), IL-4, or CD40 and triggered predominantly B-cell IgG secretion with IL-2. Even more striking levels of B-cell proliferation occurred with HB22.7 MoAb under culture conditions that enhanced B-B-cell interactions. In contrast, a nonblocking CD22 MoAb (CD22.5) poorly costimulated in similar experiments. The functional differences between the two antibodies likely result from differing abilities to trigger downstream signaling events as significant differences in CD22 tyrosine phosphorylation and the recruitment of the tyrosine kinase p53/56lyn and the tyrosine phosphatase SH-PTP1C were found. Besides their role in B-cell stimulation, CD22/CD22L interactions may also assist in regulating T-cell proliferation because inhibition of CD22/CD22L engagement with the HB22.7 MoAb impaired T-cell proliferation in a costimulatory assay. Thus, CD22/CD22L interactions result in stimulatory signals for both B and T lymphocytes.

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D22 IS a B-lymphocyte-specific membrane protein.1 It first appears in B-cell ontogeny at the late pro-B cell stage, but only intracellularly. Later in development at the immature B-cell stage its expression shifts to the plasma membrane. The mechanism that accounts for this switch from an intracellular to a membrane form is unknown. Nearly all mature B lymphocytes express CD22, although it disappears upon their differentiation to plasma cells. Although the precise function of CD22 in B-cell physiology remains unknown, considerations of the primary structure of CD22 has led to the pursuit of two productive avenues of investigation.

The first of these focused on the extracellular portion of the molecule. A member of the Ig superfamily CD22 has two different isoforms termed CD22α and CD22β that contain five and seven Ig-like domains, respectively.2,4 The CD22β isoform is the most prevalent on mature B lymphocytes and likely the most important. The similarity of the extracellular portion of CD22 to the cell adhesion molecules myelin associated glycoprotein (MAG) and carcinoma embryonic glycoprotein (CEA) suggested that CD22 might mediate cell-to-cell interactions. Consistent with this hypothesis, transfection of CD22 into COS cells conferred upon them the ability to bind monocytes, erythrocytes, neutrophils, and lymphocytes.2,3 The binding depended on the recognition of sialic acid residues in glycoconjugates on the target cells, but not on the presence of calcium or magnesium ions in the media.5,7 The CD22 ligands on the target cells will be denoted as CD22L. CD22 monoclonal antibodies (MoAbs) that block the binding of lymphocytes to CD22-transfected COS cells also block the binding of erythrocytes and neutrophils.7 These CD22-blocking antibodies bind epitopes localized in the first two Ig domains of CD22.8 The use of a recombinant fusion protein containing the three N-terminal Ig domains of CD22 identified the tyrosine phosphatase CD45 as one of several potential CD22Ls; however, the relative importance of the CD22-CD45 interaction in mediating cell adhesion remains unknown.5,9,10

The requirement of CD22 to recognize sialic acid on its target cell is a feature of several other adhesion proteins including MAG and the recently discovered protein sialoadhesin.11 Together they form the basis for a novel family of sialic acid binding proteins.12 Besides their structural similarities, the genes that encode these proteins all map to chromosome 19q in humans and to the syntenic region on chromosome seven in mice.13,14 This region of the human genome also contains CEA and a cluster of CEA-related genes.15 As noted above, CEA and many of the CEA-related genes also share some homology with CD22.

The other avenue of investigation that arose from analysis of the primary structure of CD22 focused on its intracellular portion. Within it are six tyrosines, four in two homologous tyrosine motifs.16,17 Phosphorylation of the tyrosines within an ITAM motif facilitates its interactions with the SH-2 domains of effector molecules. Several important lymphocyte signaling molecules contain this motif including the intracellular portions of the B-cell antigen receptor-associated molecules mb-1 and B29, and the T-cell antigen receptor associated molecules CDx, CD3ε, and CD3γ.18,19 Several pieces of evidence suggest that these ITAM-like regions in CD22 are functionally significant. Engagement of the B-cell antigen receptor or direct engagement of CD22 with an MoAb triggers CD22 tyrosine phosphorylation.20-22 Likely because of this tyrosine phosphorylation increased amounts of p72syk, p85PI-3 kinase, and p53/p561yn associate with CD22.23-25 The use of tyrosine-phosphorylated synthetic peptides mapped the region of CD22 that likely mediates the associations of p53/56lyn and p72syk to the distal ITAM-like motif (amino acids 822 to 842).26

In this study, we show that engagement of CD22 with an
MoAb that blocks the interaction between CD22 and CD22L potently stimulates B lymphocytes and augments signals delivered via the B-cell antigen receptor complex, CD40, and two cytokine receptors. In addition, the availability of the CD22 blocking MoAb allowed a direct test of the potential importance of CD22/CD22L interactions in T-cell activation/proliferation.

MATERIALS AND METHODS

Antibodies and flow cytometry. The MoAb HB22.7 (y2wK) and CD22.5 (HD239, yzw) Fifth International Workshop on Human Leu-
kocyte Differentiation Antigens, Boston, MA) have been previously described.14 Both antibodies interact with the N-terminal Ig domains of CD22, but CD22.5 does not bind in a ligand-binding region. The antibodies gave similar results when used for immunofluorescent staining of tonsil B cells or B-cell lines (data not shown). The CD22 polyclonal antiserum was prepared in rabbits using keyhole limpet hemocyanin conjugated with the peptide AWENVDYNILKH, whose sequence was derived from the COOH-terminus of human CD22. The CD3 MoAb was a kind gift of Dr Scott Koenig (Medim-
mune, Gaithersburg, MD). The H1F6 MoAb was used as an isotype-
matched control for the Ig production assays. This antibody is a purified MoAb produced against activated B cells and does not significantly affect B-cell proliferation or Ig production (J. Tuscano, et al, manuscript in preparation). Purity of tonsil B cells was monitored by CD20 immunofluorescence (CD20-FITC; Becton Dickinson Immunocytochemistry Systems, Mountain View, CA) using single-
color cytofluorometry on an EPICS profile I (Coulter, Hialeah, FL).

Cell culture and assays. Normal B and T lymphocytes were isolated from human tonsils. The tonsil B cells and T cells were purified as previously described.22 The RAMOS cell line was obtained from the American Type Culture Collection (ATCC; Rock-
sville, MD) and maintained in continuous culture in RPMI 1640 plus
10% fetal calf serum (FCS; GIBCO-BRL, Gaithersburg, MD). Tonsil B cells were cultured in 96-well flat-bottom microtiter plates (Costar, Cambridge, MA) at a concentration of 100,000 cells/well in RPMI 1640 supplemented with 10% FCS at 37°C in a 5% CO2 atmosphere. B cells were stimulated with various combinations of CD40 MoAb (G2B-5 MoAb, kindly provided by Dr E. Clark, University of Washing-
ton, Seattle, WA), anti-GME (IL-4, Genzyme, Cambridge, MA), IL-2 (Cetus Corp, Emeryville, CA), anti-IgM (4B8 MoAb, kindly provided by Dr E. Clark), HB22.7, or CD22.5 MoAb. Each experimental condition was done in triplicate. B cells were cultured for 3 days and 1 μCi [3H]-thymidine was added over the last 18 hours of the culture period. The cells were procured and thymidine incorporation into DNA measured by standard scintillation counting. Similar cultures were performed in 96-well round-bottom plates (Costar) and assayed for thymidine incorporation as described above. Ig secretion was assessed by measuring IgG and IgM levels in culture supernatants derived from B-cell cultures established similarly to those used for measuring B-cell proliferation except that the cultures were allowed to continue for 7 days. The IgG and IgM levels were determined by ELISA as previously described.15 Several of the prol-
iferation assays were also done in the presence of CDw32-
transfected L cells as previously described.17 The costimulation assays were performed as described.16 Briefly, purified tonsil B cells were stimulated with Staphylococcus aureus Cowan strain 1 (SAC) for 24 hours, washed, and irradiated with 5,000 rads. The stimulated and irradiated tonsil B cells (200,000 cells/well) were cocultured with tonsil T cells (300,000 cells/well) in the presence of CD3 MoAb (1:10,000 dilution of ascites) in the presence or absence of HB22.7 or CD22.5 MoAb. The cultures were allowed to proceed for 3 days before measuring thymidine incorporation into DNA as described above.

Immunoprecipitations and immunoblotting. RAMOS cells (2 × 106) or tonsil B cells (2 × 106) were incubated with HB22.7 or CD22.5 MoAb (10 μg/mL) for 1 or 5 minutes. The antibodies were crosslinked with 8 × 105 Dynabeads (Dynal Corp, Oslo, Norway), the cells pelleted and then resuspended in 1% digitonin (wt/vol) lysis buffer (Sigma Chemical Co, St Louis, MO), 50 mmol/L Tris-
HCl (pH 8.0), 150 mmol/L NaCl, 0.5 mmol/L EDTA, 2 mmol/L orthovandate (Sigma), 2 μg/mL aprotinin, 2 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride (Boehringer Mannheim, Indianapolis, IN) and kept overnight at 4°C. After lysis, HD39 MoAb (5 μL, Boehringer Mannheim) was added to each reaction to ensure adequate CD22 antibody for immunoprecipitation. Immunoprecipi-
tates were isolated and washed five times with digitonin lysis buffer. The immunoprecipitates were size fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and trans-
ferred to nitrocellulose. The filters were blocked with TTBS (150 mmol/L NaCl, 50 mmol/L Tris-HCl [pH 7.4], 0.05% Tween 20, and 10% milk or 3% bovine serum albumin [BSA]). The immunoblots were then incubated with the antiphosphotyrosine MoAb 4G10 (Up-
state Biotechnology Inc, Lake Placid, NY) at a dilution of 1:1,000 in TTBS and 1% BSA.

The blot was stripped and sequentially probed with rabbit anti-
SH-PTPI (Upstate Biotechnology) and rabbit anti-p53/p56lyn (Up-
state Biotechnology) using the manufacturer’s recommendations. CD22 levels in the immunoprecipitates were assayed by immu-
no blotting with the rabbit anti-CD22 antibody derived against a C-
terminal peptide (1:200 dilution of antisera). All the immunoblots were reacted with a goat-antimouse or sheep-antimouse antibodies conjugated to HRP (Amersham, Buckinghamshire, UK). All signals were detected by enhanced chemiluminescence (ECL, Amersham).

RESULTS

Engagement of CD22 induces tonsil B-cell proliferation that is augmented by cytokines or CD40. Several CD22 MoAbs block the interaction of CD22 with its ligand(s) on lymphocytes, erythrocytes, or neutrophils.5,6 In the present experiments we compared the abilities of two isotype-
matched MoAbs, HB22.7 (a blocking MoAb) and CD22.5 (a nonblocking MoAb), to directly stimulate B-cell proliferation and to costimulate B-cell proliferation with cytokines or CD40. The addition of the HB22.7 MoAb to B-cell cultures increased thymidine incorporation approximately sixfold over background while the addition of the CD22.5 MoAb had no appreciable effect (Fig 1). In the cytokine costimula-
tory experiments, the addition of 10 U of IL-2 significantly enhanced the response to HB22.7 (approximately threefold above HB22.7 alone), but not to CD22.5. A small costimula-
tory effect with CD22.5 and IL-2 occurred at the higher concentrations of IL-2 tested (Fig 2A). HB22.7 and 100 U/mL of IL-4 also costimulated B-cell proliferation (approxi-
mately twofold above HB22.7 alone), although less efficiently than did HB22.7 and IL-2 (Fig 2B). IL-10 failed to costimulate with either HB22.7 or CD22.5 MoAb (data not shown). In an attempt to more efficiently crosslink CD22 and to reduce the potential for Fc receptor binding, several assays were done in the presence of CDw32-transfected L cells. This resulted in a marginal (~10% to 20%) increase in proliferation compared to the B cell stimulated with HB22.7 alone (data not shown).

Because the interaction of CD40 with CD40 ligand is
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CD22.5 is much less stimulatory either directly or in various costimulation assay.

HB22.7 MoAb and anti-Ig costimulate B-cell proliferation, and culturing B cells under conditions that enhance B-B-cell interactions increases the response to HB22.7 MoAb. Stimulation of B cells with antigen in conjunction with costimulatory signals provided by T cells initiates the humoral immune response. Anti-Ig has been used to mimic the antigen signal and previously shown to weakly costimulate with HD6. We compared HB22.7 and CD22.5 in a costimulatory assay with an anti-IgM MoAb. Although both CD22 MoAbs costimulated tonsil B-cell proliferation, HB22.7 was clearly superior (approximately threefold better). The amount of proliferation achieved with anti-Ig and HB22.7 reached levels similar to those observed with IL-2 and HB22.7 (Fig 3A).

All the previously described experiments used flat-bottom culture plates to reduce cell-cell interactions. To determine whether increased B-cell to B-cell interactions might modulate the response to the HB22.7 MoAb, we cultured purified tonsil B cells in round bottom plates in the presence of similar concentrations of HB22.7 or CD22.5 MoAb. Not surprisingly, the background level of thymidine incorporation increased in the round-bottom plate cultures. While blocking any potential CD22/CD22L interactions by adding the MoAb HB22.7 to the round-bottom culture wells, we simultaneously stimulated B lymphocytes by engaging CD22. The overall result was a significant augmentation in B-cell proliferation (Fig 3B). The fold increase in thymidine incorporation over background was similar in the round- and flat-bottom plates. CD22.5 had little effect under either culture condition (Fig 3B).

Stimulation of tonsil B cells with MoAb HB22.7 and IL-2 induces Ig production. Stimulation of human B cells with either CD40 or anti-Ig does not induce Ig secretion unless a second signal such as IL-2 or IL-10 is present. Similarly, stimulation of human B cells with HB22.7 MoAb alone or with a CD40 MoAb resulted in no significant increase in Ig secretion above the low baseline levels detected in culture supernatants (data not shown). However, after the addition

![Fig 1. Engagement of CD22 results in increased B-cell proliferation. Purified tonsil B cells were cultured with increasing concentration of either HB22.7 (○) or CD22.5 (●) and the amount of [H]-thymidine incorporated between 56 and 72 hours of culture was measured. The experiment was repeated a minimum of three times. Results are from a representative experiment. Triplicate values varied less than 10%.](image)

![Fig 2. IL-2, IL-4, and CD40 augment CD22 induced B-cell proliferation. Increasing concentration of recombinant IL-2 (A), IL-4 (B), or CD40 MoAb (C) added to either HB22.7 (10 μg/mL) or CD22.5 (10 μg/mL). (○), HB22.7; (●), CD22.5; (△), media with increasing amounts of IL-2 (A), IL-4 (B), or CD40 (C). The incorporation of [H]-thymidine was measured between 56 and 72 hours of culture. Each experimental condition was repeated a minimum of three times. Results are from representative experiments. Triplicate values varied less than 10%.](image)
HB22.7 costimulates with anti-Ig and further augments B-cell proliferation under conditions that enhance B–B-cell interactions. Tonsil B cells were stimulated with increasing concentrations of HB22.7 (O), CD22.5 (□), anti-IgM MoAb (△), or costimulated with anti-IgM (10 μg/mL) and increasing concentrations of HB22.7 (○), or anti-IgM (10 μg/mL) and increasing concentrations of CD22.5 (●). The x-axis is increasing concentrations of either HB22.7, CD22.5, or anti-IgM. Results are representative of one of two experiments performed (A). Tonsil B cells were also cultured in either round- or flat-bottom plates with media alone, HB22.7 (10 μg/mL), or CD22.5 (10 μg/mL) (B). [H]-Thymidine incorporation was measured between 56 and 72 hours of culture. Results are representative of one of three experiments performed. Triplicate values varied less than 15%.

HB22.7 and CD22.5 differ in their abilities to trigger CD22 tyrosine phosphorylation and the association of CD22 with p53/56lyn and SH-PTP1C. The marked functional differences between the two CD22 antibodies prompted us to compare their abilities to trigger CD22 tyrosine phosphorylation and to trigger the association with downstream effectors. Purified tonsilar B cells or RAMOS cells, a B-cell line previously used to study signal transduction through CD22, were treated with HB22.7 or CD22.5 MoAb immobilized on goat-antimouse Ig magnetic beads. At various time points we immunoprecipitated CD22, size fractionated the immunoprecipitates via SDS-PAGE, and immunoblotted with an antiphosphotyrosine MoAb. HB22.7 MoAb stimulated more CD22 tyrosine phosphorylation than did CD22.5 MoAb in purified tonsilar B cells (Fig 5A). Stripping and reprobing with anti-CD22 polyclonal antisera prepared with a C-terminal peptide verified that all immunoprecipitates contained nearly equivalent levels of CD22 (Fig 5B). In similar experiments with RAMOS cells, HB22.7 stimulated more tyrosine phosphorylation of CD22 than did CD22.5 (Fig 6). Next, we examined for the presence of two downstream effectors known to associate with CD22, the src kinase p53/56lyn and the tyrosine phosphatase SH-PTP1C. Reprobing the same filter with antisera against anti-p53/56lyn or SH-PTP1C showed higher levels of both proteins in the CD22 immunoprecipitates prepared from HB22.7 stimulated cells than from the CD22.5 stimulated cells (Fig 6). Again, stripping and reprobing with an anti-CD22 antisera verified that the immunoprecipitates contained nearly equivalent levels of CD22 (Fig 6). Although these experiments were performed using digitonin lysates, similar results were found with NP-40 lysates (data not shown).

Blocking CD22/CD22L interactions inhibits T-cell proliferation. Many different cell-surface molecules can provide a costimulatory signal for T-cell proliferation. The availability of the HB22.7 MoAb allowed us to test whether blocking the interaction of CD22 with CD22L altered T-cell proliferation. Irradiated, activated human B lymphocytes plus CD3 MoAb stimulated high levels of T-cell proliferation as assessed by [H]-thymidine incorporation. The addition of the blocking MoAb HB22.7 reduced the level of

![Fig 3](image-url)  
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![Fig 4](image-url)  
Fig 4. Ligation of CD22 induces B-cell Ig production in the presence of IL-2. Tonsil B cells were cultured with MoAb HB22.7 (10 μg/mL), MoAb CD22.5 (10 μg/mL), or MoAb H1F6 (10 μg/mL) in the presence or absence of recombinant IL-2 (100 U/mL). Seven days after initiation of the culture, culture supernatants were harvested and assayed for IgM (A) or IgG (B) by enzyme-linked immunosorbent assay. Results are from one of three experiments performed.
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Fig 5. Phosphotyrosine immunoblot of CD22 immunoprecipitates from purified tonsilar B cells stimulated with HB22.7 or CD22.5 MoAb. Tonsilar B cells were stimulated with HB22.7 or CD22.5 for 1 or 5 minutes in the presence of goat-antimouse Ig antibodies coupled to magnetic beads. The cells were obtained, cell lysates prepared, and CD22 immunoprecipitated. (A) The immunoprecipitates were size fractionated by SDS-PAGE and immunoblotted with an antiphosphotyrosine antibody. (B) To ensure equal loading the same blot was stripped and reprobed with anti-CD22 antisera. CD3-stimulated cells immunoprecipitated with CD3 are shown as a negative control.

CD22 crosslinking triggers an amplifiable stimulatory signal for B lymphocytes. The CD22 MoAb HB22.7 directly stimulated B-cell proliferation, costimulated B-cell proliferation with anti-Ig, cytokines, or CD40, and induced B-cell Ig production with IL-2. The HB22.7 MoAb that blocks the interactions between CD22 and its ligand(s) had potent agonist activity while the CD22.5 MoAb that does not block had significantly less activity. These functional differences correlated with differences in amount of CD22 tyrosine phosphorylation induced and the association of p53/56lyn and SH-PTP1C with CD22.

CD22-CD22L interactions likely occur during primary thymidine incorporation 25% to 30% in four separate experiments (Fig 7). In contrast, the nonblocking MoAb CD22.5 failed to modulate thymidine incorporation. The addition of either antibody to CD3 MoAb-stimulated T cells in the absence of B cells did not alter thymidine incorporation (data not shown). These data provided evidence that blocking CD22/CD22L interactions impairs T-cell activation/proliferation.

DISCUSSION

CD22 crosslinking triggers an amplifiable stimulatory signal for B lymphocytes. The CD22 MoAb HB22.7 directly stimulated B-cell proliferation, costimulated B-cell proliferation with anti-Ig, cytokines, or CD40, and induced B-cell Ig production with IL-2. The HB22.7 MoAb that blocks the interactions between CD22 and its ligand(s) had potent agonist activity while the CD22.5 MoAb that does not block had significantly less activity. These functional differences correlated with differences in amount of CD22 tyrosine phosphorylation induced and the association of p53/56lyn and SH-PTP1C with CD22.

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Fig 6. Immunoblotting of CD22 immunoprecipitates from RAMOS cells stimulated with HB22.7 or CD22.5 MoAb. A similar experiment as described in Fig 5 with RAMOS cells. The immunoblot was sequentially probed with an anti-phosphotyrosine MoAb, an SH-PTP1C antiserum, a CD22 polyclonal antiserum, and a p53/56lyn antiserum.

Fig 7. Blocking a CD22/CD22L interaction impairs T-cell proliferation. Tonsil B cells were activated with SAC for 2 days, irradiated, and added to purified T cells in the presence of a CD3 MoAb (control), in the presence of CD3 MoAb and CD22.5 MoAb (10 μg/mL), or in the presence of CD3 MoAb and HB22.7 MoAb (10 μg/mL). The amount of [3H]-thymidine incorporated was measured 3 days later. The results are presented as the mean + 2 SD of four experiments. Each experimental point derived from a triplicate culture. In each of the control experiments the T cells incorporated approximately 200,000 cpm.
and secondary immune responses where T–B-cell and perhaps B–B-cell interactions are critical. The adhesive properties of CD22 undoubtedly contribute to these cell-cell interactions. Facilitating contact between B and T cells likely enhances contact between B-cell costimulatory molecules such as B7-1 and B7-2 with their counter-receptors on T cells. Although an important component of a CD22-CD22L interaction for T cells may be attributed to the adhesive properties of CD22, a significant aspect of this interaction for B cells is the activation of the CD22 signal transduction pathway. Triggering this pathway may amplify signals provided via interactions between antigen/antigen receptors, CD40L/CD40, and cytokine/cytokine receptors. Thus, triggering the CD22 signal transduction pathway may be functionally important at several distinct phases of B-cell differentiation. It may participate in B-cell activation via costimulation with antigen. Such costimulatory signals (antigen/antigen receptor and CD22L/CD22) could occur after initial exposure to antigen, during the course of T–B-cell interactions, or within the germinal center region when B cells interact with antigen associated with follicular dendritic cells.

Engagement of CD22 may also be important in the clonal expansion of B cells, particularly in conjunction with cytokines and/or CD40L. In a thymus-dependent immune response, antigen-activated B cells migrate into a primary lymphoid follicle where they rapidly proliferate in a confined area producing the dark zone of a germinal center. The identities of the growth stimulatory signals that fuel this rapid B-cell expansion in the dark zone are unknown, and it is possible that CD22-CD22L interactions contribute a growth stimulatory signal in this locale. The finding that engagement of CD22 with the HB22.7 MoAb significantly enhanced the absolute level of [³H]-thymidine incorporation in round-bottom compared with flat-bottom plates suggests that activation of the CD22 signal transduction pathway amplifies stimulatory signals resulting from the close physical approximation of B cells, a situation that occurs in the dark zone. Finally, although likely important in the activation and expansion of B lymphocytes, CD22 disappears as B cells differentiate into plasma cells, indicating that the CD22 signal transduction pathway does not have a significant role during the terminal stages of B-cell differentiation.

The superiority of the HB22.7 versus CD22.5 MoAb in stimulating or costimulating B-cell proliferation likely results from differing abilities to trigger CD22 tyrosine phosphorylation and to recruit downstream effectors. We previously showed that p53/56lyn associates with CD22 following CD22 crosslinking and here show that SH-PTP1C also rapidly associates. Considerable evidence supports a critical role for p53/56lyn in B-lymphocyte signal transduction pathways. Besides its association with the CD22, it also has been found to associate with the B-cell antigen receptor complex and with the CD19/CD21 complex. In contrast to the positive effects of p53/56lyn, recent evidence indicates that SH-PTP1C negatively regulates signal transduction pathways. However, despite its more potent stimulatory effects, HB22.7 crosslinking resulted in more SH-PTP1C associated with CD22 than did CD22.5 crosslinking. These data suggest that a balance between the positive and negative effectors recruited dictates the outcome of receptor engagement (see below).

Several recent reports have shown that SH-PTP1C associates with CD22 after crosslinking of the antigen receptor. SH-PTP1C contains two SH-2 domains at its amino terminus and a carboxyl terminus tyrosine phosphatase catalytic domain. The recognition that motheaten (me) and viable motheaten (vme) mice have loss-of-function mutations in this gene showed the importance of SH-PTP1C in hematopoiesis and immune function. The deficient mice develop a progressive inflammatory disease with abnormal hematopoiesis and elements of both immunodeficiency and autoimmunity. SH-PTP1C associates with and may dephosphorylate the erythropoietin, c-kit, and IL-3 receptors. In addition, coligation of the Fcγ RIIB1 with the B-cell antigen receptor complex leads to abortive signaling by recruiting SH-PTP1C into the complex. A likely role for SH-PTP1C is to counteract the positive effects of Src and Jak family kinases that also associate with these receptors. Similarly, the association of SH-PTP1C with CD22 likely counteracts the positive effects of p53/56lyn, and perhaps p72syk and p85PI-3 kinase. Consistent with this idea, we found that p53/56lyn, p72syk, and SH-PTP1C preferentially bind to a tyrosine phosphorylated peptide that spans the distal ITAM-like region in CD22, suggesting that tyrosine phosphorylated CD22 may be a substrate for SH-PTP1C resulting in dephosphorylation and receptor recycling (J. Tuscano, unpublished observation, October 1995). There is evidence to suggest that CD22 negatively regulates signaling via the antigen receptor while our results indicate that engagement of CD22 can provide a stimulatory signal to B cells. Together these results suggest that engagement of the antigen receptor in the absence of CD22 crosslinking may impair signal transduction while stimulation of the antigen receptor in the context of CD22-CD22L interactions augments signaling.

We have also shown that inhibition of CD22-CD22L engagement partially impairs T-cell activation in a costimulatory assay using CD3 MoAb and irradiated B cells. This contrasts with previously published experiments using a CD22 fusion protein that demonstrated costimulation of T cells with CD3 MoAb and the fusion protein suppressed T-cell proliferation. Furthermore, crosslinking CD22L using the CD22 fusion protein blocked CD3 MoAb-induced increases in intracellular calcium and phosphorylation of phospholipase Cγ1. From these previous experiments, the inhibition of a CD22/CD22L interaction would have been predicted to enhance T-cell activation, rather than inhibit it as we observed. Although there are several potential explanations for these differences, perhaps the most likely is that the MoAb blocking experiments reported in this study are in the context of cell-cell interactions whereas the fusion protein experiments only examine the consequences of crosslinking CD22Ls and CD3. The engagement of other surface molecules such as CD2-LFA-1 and B7-CD28 may significantly alter the consequences of the CD22L-CD22 interaction. Indeed, the inhibitory effect of HB22.7 on T-cell costimulation may result from reducing B–T-cell collaboration by decreasing interactions with other cell-surface stimulatory
molecules. However, consistent with the ability of CD22L to transmit a positive signal to T cells, a more recent set of experiments demonstrated an augmented intracellular calcium response to CD3 MoAb stimulation in the presence of the CD22 fusion protein.52

In conclusion, we have shown that engagement of CD22 on B cells augments the effects of a variety of other stimulatory signals including anti-Ig, CD40, and IL-2. These experiments used an MoAb known to block the interaction of CD22 with CD22L that results in cell adhesion. In contrast, a nonblocking CD22 MoAb poorly stimulated and less efficiently induced CD22 tyrosine phosphorylation and the recruitment of downstream effectors such as p53/56lyn and SH-PTP1C. Finally, we provided evidence that the interaction of CD22 with CD22L on T cells enhances T-cell activation/proliferation. This augmentation may result from the adhesive properties of CD22L or via direct signaling through CD22Ls.

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