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Regulation of CD45 engagement by the B-cell receptor CD22

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ABSTRACT **The B-cell receptor CD22 binds sialic acid linked α-2–6 to terminal galactose residues on N-linked oligosaccharides associated with several cell-surface glycoproteins. The first of these sialoglycoproteins to be identified was the receptor-linked phosphotyrosine phosphatase CD45, which is required for antigen/CD3-induced T-cell activation. In the present work, we examine the effect of interaction between the extracellular domain of CD45 and CD22 on T-cell activation. Using soluble CD22-immunoglobulin fusion proteins and T cells expressing wild-type and chimeric CD45 forms, we show that engagement of CD45 by soluble CD22 can modulate early T-cell signals in antigen receptor/CD3-mediated stimulation. We also show that addition of sialic acid by β-galactoside α-2,6-sialyltransferase to the CD22 molecule abrogates interactions between CD22 and its ligands. Together, these observations provide direct evidence for a functional role of the interaction between the extracellular domain of CD45 and a natural ligand and suggest another regulatory mechanism for CD22-mediated ligand engagement.**

CD22 is a B-cell-specific receptor, structurally related to several immunoglobulin superfamily adhesion molecules, including myelin-associated glycoprotein and cellular adhesion molecules ICAM-1, VCAM-1, and NCAM (1–3). At least two isoforms, CD22α and CD22β possessing an extracellular region composed of five and seven immunoglobulin subdomains respectively, can be expressed in B cells (1–3). All known functional properties of CD22 are displayed by CD22β, which appears the dominant isoform expressed on B cells and will henceforth be referred to as CD22. Normal and neoplastic T and B cells can adhere to COS cells transfected with CD22 cDNA (3), and several T- and B-cell surface sialoglycoproteins can be immunoprecipitated using a soluble CD22-immunoglobulin (CD22 receptor globulin; CD22Rg) fusion protein (4, 5). These observations led to the definition of the natural ligand of CD22 as sialic acid linked α-2–6 to the sequence Gal(β1-4)GlcNac, an acceptor disaccharide commonly associated with N-linked oligosaccharides (5, 6). Addition of sialic acid to this receptor disaccharide is mediated by a β-galactoside α-2,6-sialyltransferase (α-2,6ST) (7), and affinity of CD22 for its glycoprotein ligands is determined, in part, by the number of α-2,6-linked sialic acid residues on each oligosaccharide side chain (6), the number of α-2,6-linked sialic acid-containing oligosaccharide side chains (8), and appropriate oligosaccharide clustering and presentation by the underlying protein backbone (6, 8). Interestingly, the earliest CD22-binding cell-surface sialoglycoprotein to be identified was CD45, the leukocyte-specific receptor-linked phosphotyrosine phosphatase (4, 5, 9).

CD45 is thought to couple the antigen-receptor complex to intracellular signaling pathways, and its expression is therefore critical for antigen-receptor-mediated activation of both T and B lymphocytes (10–13). This hypothesis is supported by observations that normal activation signals cannot be induced by stimulation of the antigen-receptor/CD3 complex in CD45-deficient T-cell mutants but that early signal-transduction events are restored by reintroduction of the CD45 molecule (13–15). Crosslinking of CD45 with various receptors on T cells and T-cell lines, including the antigen-receptor/CD3 complex, CD2, and CD4, using monoclonal antibodies (mAbs), may enhance or inhibit antigen-receptor/CD3-induced lymphocyte activation (16), suggesting that aggregation of CD45 with antigen-receptor complex and associated molecules may play an important regulatory role in T-cell activation. Structural heterogeneity of the extracellular domain of CD45 (10) suggests possible interactions with ligands that might facilitate CD45 aggregation with the antigen-receptor complex or directly stimulate intracellular phosphotyrosine phosphatase activity (13, 15).

Results from recent studies have suggested that CD22–CD45 interaction might operate in lymphocyte activation, based on the observation that crosslinking of anti-CD3 mAb and CD22Rg on a T-cell line inhibits early T-cell activation signals, mimicking the effect of crosslinking CD3 and CD45 with mAbs (4). However, interaction between CD22 and additional T-cell glycoproteins precluded the conclusion that the inhibition was due exclusively to CD45–CD22 association. We now show that the CD22Rg-induced modulation of early T-cell activation events depends upon physical interaction between CD22 and CD45 and provide direct evidence that engagement of CD45 by a natural extracellular ligand can trigger activation regulatory signals. Furthermore, we show that the presence of α-2,6-linked sialic acid on CD22Rg inhibits CD22–ligand interaction and abrogates CD22Rg-mediated augmentation of T-cell activation. Receptor and ligand sialylation in α-2,6-linkage may therefore be a mechanism that regulates CD22–CD45 binding and the subsequent responses.

MATERIALS AND METHODS

Immunoprecipitation and Immunoblotting. Jurkat and J45/CH11 cells (15) were surface-iodinated with ^{125}I (ICN) and lysed in phosphate-buffered saline (PBS)/0.5% Nonidet P-40/aprotinin at 20 μg/ml (Sigma). Lysates were precleared with protein A–Sepharose beads (Repligen) for 2 hr at 4°C and incubated with anti-CD45 mAb 9.4 at 4 μg/ml (4), CD22Rg at 80 μg/ml (3, 17), or control receptor globulin, CD5Rg or CD44Rg (80 μg/ml), and fresh protein A–Sepharose beads overnight at 4°C. Protein A beads were washed three times in PBS/0.05% Nonidet P-40, and immunoprecipitates were eluted by boiling in sample buffer and subjected to SDS/5–10% PAGE under reducing conditions. Gels were fixed, dried,

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Abbreviations: mAb, monoclonal antibody; CD22Rg, CD22 receptor globulin; NCV, Newcastle disease virus; *Au*, *Arthrobacter ureafasciens*; α-2,3-sCD22Rg, α-2,3-sialyltransferase-modified CD22Rg; α-2,6-sCD22Rg, α-2,6-sialyltransferase-modified CD22Rg; α-2,6ST, α-2,6-sialyltransferase; LFA3Rg, LFA3 receptor globulin; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration.

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and autoradiographed (4-day exposure). For immunoblotting, immunoprecipitates were electrotransferred onto nitrocellulose filters (Amersham) by using a Bio-Rad Trans-Blot apparatus. Filters were blocked with PBS/5% skim milk (Difco)/0.5% fetal bovine serum (Irvine Scientific) for 8 hr at 4°C, washed three times with PBS/0.02% Tween 20 (Sigma), and incubated with anti-CD45 mAb 9.4 at 1 μg/ml overnight at 4°C. Immunoreactive proteins were identified after incubation with either horseradish peroxidase-conjugated goat anti-mouse antibody (Amersham) at a 1:50,000 dilution for 1 hr at room temperature and revelation by autoradiography with an enhanced chemiluminescence detection system (Amersham) according to the recommended protocol. Filters were autoradiographed for 2–3 min.

Phosphorylation of Phospholipase Cγ1 (PLCγ1) Assay. Cells (1×10^6) were incubated at room temperature in medium or medium with anti-CD3 alone at 0.34 μg/ml (for Jurkat cells) or 1.6 μg/ml (for CH11 cells) (provided by Jeff Ledbetter, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle) or with anti-CD3 and CD22Rg, LFA3 receptor globulin (LFA3Rg), or CD44Rg at 20 μg/ml for 30 sec. Antibody and receptor globulins were then crosslinked with goat anti-human antibody at 20 μg/ml (Organon Teknika) for 90 sec, and cells were lysed in lysis buffer containing phosphatase inhibitors as described (18). Lysates were incubated with anti-PLCγ1 antibody (Upstate Biotechnology, Lake Placid, NY) (7.5 μg/ml, final concentration) and protein A–Sepharose for 5 hr at 4°C, the beads were washed in lysis buffer, and immunoprecipitated protein was eluted and separated by SDS/7% PAGE, transferred onto nitrocellulose filters (Hybond-C, Amersham), and blotted with horseradish peroxidase-conjugated anti-phosphotyrosine mAb 4G10 at 1 μg/ml (Upstate Biotechnology). Membranes were stripped and reblotted with anti-PLCγ1 antibody and horseradish peroxidase-conjugated goat anti-mouse antibody at 1 μg/ml. Proteins were revealed by enhanced chemiluminescence (6-hr exposure for anti-phosphotyrosine antibody and 30 sec–1 min for anti-PLCγ1 antibody).

Inositol Phosphate Determination. Cell lines were loaded with myo-[^{3}H]inositol (NEN/DuPont) overnight, preincubated for 30 sec with anti-CD3, anti-CD3 and CD22Rg, or anti-CD3 and CD44Rg at the same concentrations as above and crosslinked with goat anti-human antibodies for 3 min. Soluble inositol phosphates were isolated and measured as described (13).

Calcium Flux Assay. Cells were loaded with 1.0 mM indo-1 acetomethoxyester (Molecular Probes) in RPMI 1640 medium for 40 min at 37°C (19). Baseline stimulation values were established using purified anti-CD3 (4) at 0.34 μg/ml (final concentration) for Jurkat cells and 1.6 μg/ml (final concentration) for J45/CH11 cells, as above. CD22Rg and CD44Rg were added at 20 μg/ml (final concentration) at 30 sec, and goat anti-human crosslinker was added at 20 μg/ml (final concentration) for goat anti-human antibody and 60 μg/ml (final concentration) for goat anti-human F(ab′)$_2$ at 90 sec. Cells were analyzed for free Ca^{2+} by simultaneous measurement of violet and blue fluorescence emission (19); a Coulter Epics flow cytometer and multitime software (Phoenix Flow Systems, San Diego) were used.

Production of α-2,3- and α-2,6-Sialylated Receptor Globulins. cDNAs (50 μg each) encoding CD22Rg and α-2,3- (20) or α-2,6ST (17) were cotransfected into COS cells by the DEAE-dextran method, and receptor globulins were harvested and purified on protein A–Sepharose as described (3, 4, 9). Aliquots of protein A–Sepharose-bound receptor globulins were subjected to either *Arthrobacter ureafaciens* (*Au*) sialidase at 20 milliunits per ml or Newcastle disease virus (NDV) sialidase at 20 milliunits/ml (Boehringer Mannheim) for 1 hr at 37°C and washed three times with PBS before elution. Sialidase-treated and -untreated receptor globulins were analyzed by SDS/5–10% PAGE and then stained with silver nitrate.

RESULTS

Interaction of CD22Rg with CD45 on Wild-Type and Mutant Human T-Cell Lines. To determine whether CD22–CD45 interaction might be significant in lymphocyte signaling, we used wild-type Jurkat T cells and a CD45-deficient Jurkat-derived mutant, J45/CH11, expressing a recombinant fusion protein composed of the CD45 intracellular domain and the extracellular and transmembrane domains of the major histocompatibility complex class I molecule A2 (13). Expression of the chimeric A2–CD45 molecule restored T-cell receptor-mediated signaling in CD45-deficient Jurkat cell mutants (13), and crosslinking of A2 and CD3 with mAb blocked anti-CD3 mAb-induced activation (data not shown). Thus, J45/CH11 cells display T-cell receptor/CD3-mediated signaling comparable to that of wild-type Jurkat cells (13). However, stimuli that require engagement of the CD45 extracellular domain should be ineffective in these cells, which therefore provide a means to assess the functional implications of CD22–CD45 interaction. Glycoproteins immunoprecipitated with CD22Rg from lysates of ^{125}I-radiolabeled Jurkat and J45/CH11 cells were similar, except for two molecules of 190 and 220 kDa present only in lysates from the parental Jurkat line. These two species comigrated with the 190- and 220-kDa isoforms of CD45 immunoprecipitated from wild-type Jurkat cell lysates with anti-CD45 mAb 9.4, which recognizes all CD45 isoforms (Fig. 1*A*) and specifically reacted with mAb 9.4 on immunoblots (Fig. 1*B*). CD44Rg control precipitates showed no mAb 9.4 reactivity (Fig. 1*B*). As expected, CD22Rg did not immunoprecipitate the A2–CD45 fusion protein (data not shown).

Engagement of CD45 by CD22Rg Modulates Early T-Cell Activation Events. The functional relevance of the CD22–CD45 interaction was examined by comparing the effect of crosslinking anti-CD3 mAb and CD22Rg on CD3-induced activation of Jurkat and J45/CH11 cells. One of the earliest events in T-cell activation after stimulation by antigen or anti-receptor/CD3 mAb is tyrosine phosphorylation of phosphatidylinositol-specific PLCγ1 (21, 22), which increases phosphatidylinositol turnover (23), resulting in activating protein kinase C and increasing intracellular calcium concentration $[Ca^{2+}]_i$ (23). Protein kinase C and calcium, in turn, induce rapid activation of several secondary signal transducers. Crosslinking of CD3 with subthreshold amounts of anti-CD3

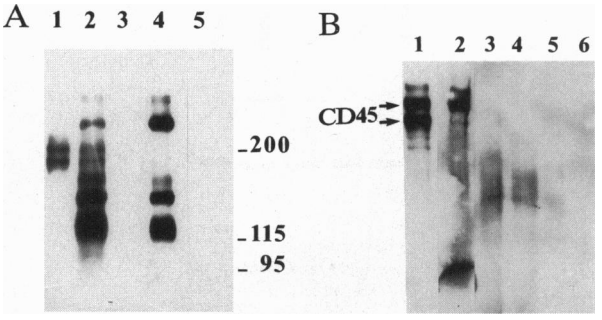

FIG. 1. (*A*) Immunoprecipitation of lysates from ^{125}I-radiolabeled Jurkat and J45/CH11 cells with anti-CD45 mAb, CD22Rg, and CD44Rg. Jurkat (lanes 1, 2, and 5) and J45/CH11 (lanes 3 and 4) lysates were immunoprecipitated with anti-CD45 mAb 9.4) (lanes 1 and 3), CD22Rg (lanes 2 and 4), and CD44Rg (lane 5). (*B*) Immunoblotting of Jurkat and J45/CH11-derived precipitates with anti-CD45 mAb. Lysates from Jurkat (lanes 1, 2, and 5) and J45/CH11 (lanes 3, 4, and 6) cells were immunoprecipitated with anti-CD45 mAb 9.4 (lanes 1 and 3), CD22Rg (lanes 2 and 4), and CD44Rg (lanes 5 and 6). Immunoprecipitates (1×10^6 cell equivalents per lane) were blotted with mAb 9.4, directed against all isoforms of CD45.

mAb alone on wild-type Jurkat cells did not detectably increase phosphorylation of PLCγ1 with respect to untreated cells (Fig. 2*A*). However, crosslinking of the same subthreshold doses of anti-CD3 with CD22Rg-augmented phosphorylation of PLCγ1 induced by anti-CD3 stimulation alone, similar to the effect of crosslinking anti-CD3 and LFA3Rg (Fig. 2*A*), a soluble immunoglobulin fusion protein form of the ligand of the T-cell costimulatory molecule CD2 (24). Coligation of anti-CD3 mAb and control receptor globulins CD44Rg (Fig. 2*A*) or CD5Rg (data not shown) on Jurkat cells did not alter anti-CD3-induced PLCγ1 phosphorylation. In contrast to the effect on wild-type Jurkat cells, crosslinking of anti-CD3 and CD22Rg on J45/CH11 cells did not augment phosphorylation of PLCγ1 (Fig. 2*C*), whereas crosslinking of anti-CD3 and LFA3Rg enhanced PLCγ1 phosphorylation in both Jurkat and CH11 cells (Fig. 2*C*).

Because phosphorylation of PLCγ1 increases production of phosphatidylinositol-derived second messengers, we examined the production of soluble inositol phosphates in Jurkat and J45/CH11 cells after stimulation with anti-CD3 or anti-CD3 crosslinked with CD22Rg. Crosslinking of anti-CD3 and CD22Rg on Jurkat cells produced significantly more soluble inositol phosphates than did anti-CD3 stimulation alone (Fig. 3*a*). Consistent with the above experiments, CD22Rg did not augment the anti-CD3-induced production of inositol phosphates in the J45/CH11 cell line (Fig. 3*b*).

The increase in PLCγ1 phosphorylation and inositol phosphate production, after crosslinking of anti-CD3 mAb and CD22Rg on Jurkat cells, should cause a corresponding increase in $[Ca^{2+}]_i$. Wild-type Jurkat cells and the J45/CH11 line were stimulated by crosslinking CD3 with mAb alone, or anti-CD3 mAb with CD22Rg, LFA3Rg, and CD44Rg, and the resulting relative $[Ca^{2+}]_i$ was measured by flow cytometry (19). Stimulation of wild-type Jurkat cells by crosslinking CD3 with mAb generated a small increase in $[Ca^{2+}]_i$ (Fig. 4). When the same cells were stimulated by crosslinking anti-CD3 and CD22Rg or LFA3Rg, a significantly greater increase in overall mean $[Ca^{2+}]_i$ was seen (Fig. 4). In contrast to LFA3Rg, however, crosslinking of anti-CD3 with CD22Rg on J45/CH11 cells, which lack the CD45 extracellular domain, did not alter the $[Ca^{2+}]_i$ increase triggered by anti-CD3 alone (Fig. 4).

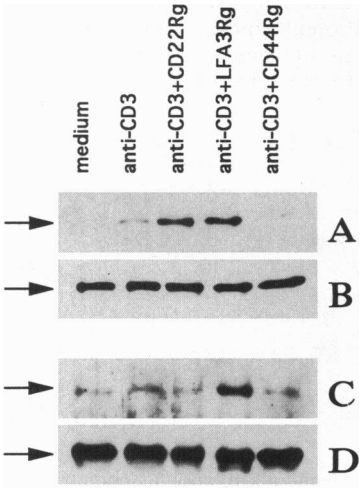

FIG. 2. Effect of CD22Rg on tyrosine phosphorylation of PLCγ1 in Jurkat (*A*) and J45/CH11 (*C*) cells. Cells (1×10^6 per lane) in *A* and *C* were treated as indicated. Ninety seconds after crosslinking, the cells were lysed, and immunoprecipitation was done with an anti-PLCγ1 antibody. Immunoprecipitates were immunoblotted with anti-phosphotyrosine antibody. Arrow, PLCγ1. Blots were autoradiographed for 6 hr. (*B* and *D*) Filters in *A* and *C* were stripped and immunoblotted with anti-PLCγ1 (arrow). Blots were autoradiographed for 30 sec–1 min.

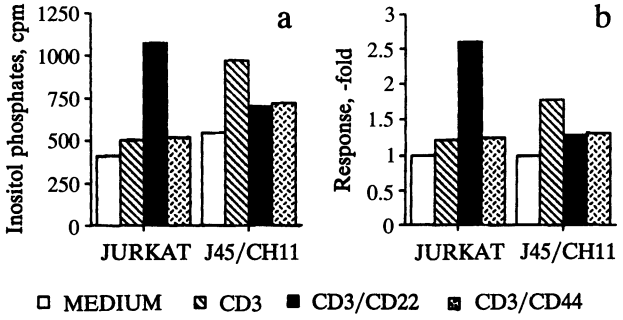

FIG. 3. Inositol phosphate generation after anti-CD3 mAb-mediated stimulation of Jurkat and J45/CH11 cells. Reagents with which anti-CD3 mAb was crosslinked are indicated. Soluble inositol phosphates are expressed in cpm (*a*) and in terms of relative increase after stimulation (*b*). Soluble inositol phosphates were isolated and measured as described (10). Data reflect a mean of triplicate experiments (SE < 10% of mean); this experiment is representative of three experiments.

CD44Rg (Fig. 4) and CD5Rg (data not shown), used at the same concentration as CD22Rg, had no effect on anti-CD3-induced change in $[Ca^{2+}]_i$ in either Jurkat or J45/CH11 cells, and crosslinking of CD22Rg alone, in the absence of CD3, induced no response in either cell type (data not shown). Taken together, our results indicate that amplification of early, anti-CD3-induced, T-cell signals resulting from anti-CD3 and

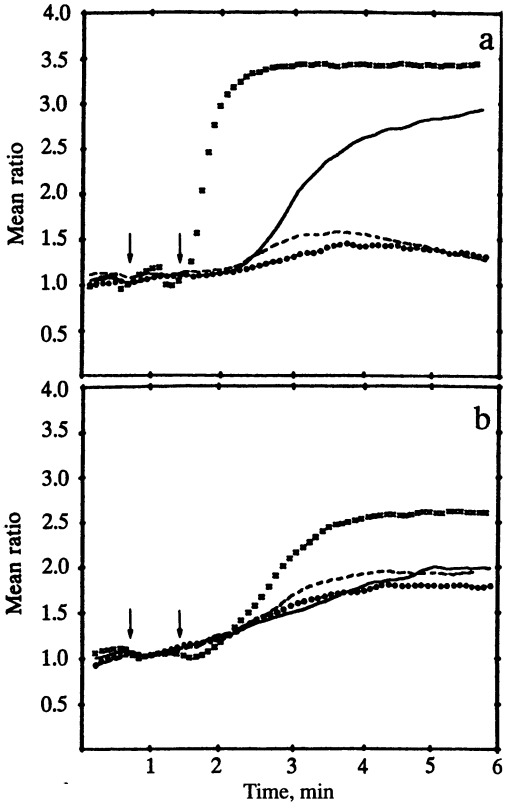

FIG. 4. Effect of CD22–CD45 interaction on anti-CD3-induced Ca^{2+} flux. CD22Rg-mediated modulation of $[Ca^{2+}]_i$ in anti-CD3-stimulated Jurkat cells (*a*) and J45/CH11 cells (*b*). ---, anti-CD3 only; •••, anti-CD3 crosslinked with CD44Rg; —, anti-CD3 crosslinked with CD22Rg; ▪▪▪, anti-CD3 crosslinked to LFA3Rg. The first arrow indicates addition of anti-CD3 mAb with or without receptor globulins, and the second arrow indicates addition of goat anti-human antibody. Cells were loaded with 1.0 mM indo-1 acetomethoxyester, and relative $[Ca^{2+}]_i$ was analyzed by measuring the violet/blue fluorescence emission ratio (395 nm:500 nm) with a Coulter Epics Elite flow cytometer. These experiments are representative of eight experiments.

CD22Rg crosslinking is a consequence of physical interaction between CD22Rg and the extracellular domain of CD45 and suggest that this interaction might lower the threshold of T-cell activation.

α-2,6-Sialylation of CD22Rg Alters Its Ability to Amplify Anti-CD3-Induced T-Cell Activation. CD22 expressed in B cells possesses N-linked oligosaccharides only, sialylated predominantly in α-2,6-linkage (25). We have recently shown that sialylation of CD22 in α-2,6-linkage abrogates its ability to mediate cell–cell adhesion (26). To address the possible regulation of CD22-dependent CD45-mediated signal transduction by CD22 sialylation, we developed α2,3- and α2,6-sialylated CD22Rg fusion proteins. Sialylation of CD22 was achieved by cotransfecting COS cells with cDNAs encoding CD22Rg with either β-galactoside α-2,6ST-specific (7, 17) or Galβ(1-3/1-4)GlcNAc α-2,3-sialyltransferase-specific (20) cDNA clones. Six days after COS cell transfection and culture in serum-free medium, supernatants were harvested, CD22Rg was purified on protein A, and fractions thereof were left untreated or treated with sialidase, as described (26). The sialidases used were derived from *Au*, which cleaves α-2,3-, α-2,6-, and α-2,8-linked sialic acid, and from NDV, which hydrolyzes α-2,3- and α-2,8-linkages (5, 6). CD22Rg produced in COS cells cotransfected with either α-2,6ST or α-2,3-sialyltransferase migrates to a slightly higher molecular weight than native CD22Rg, consistent with the hypothesis that CD22 may provide a substrate for both sialyltransferases. As expected, the molecular weight of α-2,3-sialyltransferase-modified CD22Rg (α2,3-sCD22Rg) and α-2,6ST-modified CD22Rg (α-2,6-sCD22Rg) was restored to that of native CD22Rg after NDV and *Au* sialidase treatment, respectively (data not shown).

To determine whether linkage-specific sialylation of CD22 alters its ability to bind T-cell ligands, native, α-2,3s-, and α-2,6-sCD22Rg were compared for binding to Jurkat cells by flow cytometry. Native and α-2,3-sCD22Rg bound Jurkat cell-surface ligands similarly, as demonstrated by comparable flow cytometry profiles (Fig. 5). However, binding of α-2,6-sCD22Rg was significantly weaker (Fig. 5). Pretreatment of α-2,6-sCD22Rg with *Au* sialidase partially restored binding, whereas NDV sialidase treatment had negligible effect (Fig. 5). The lack of complete restoration of binding after *Au* sialidase treatment may reflect incomplete hydrolysis of α-2,6-linked sialic acid. These observations suggest that α-2,6-sialylation of CD22Rg may inhibit its ability to modulate early T-cell activation. Calcium mobilization in Jurkat cells induced by crosslinking α-2,3-sCD22Rg with anti-CD3 mAb was identical to that induced by crosslinking anti-CD3 with native CD22Rg (data not shown), giving further support to the hypothesis that addition of sialic acid in α-2,3-linkage to CD22 has little or no effect on CD22–CD45 interaction. Crosslinking of α-2,6-sCD22Rg with anti-CD3, on the other hand, failed to augment the Ca^{2+} flux induced by anti-CD3 alone (Fig. 6). Enhancement of anti-CD3-induced Ca^{2+} flux was restored by subjecting α-2,6-sCD22Rg to *Au* but not to NDV sialidase hydrolysis (Fig. 6). That the loss of CD22Rg function was due to a steric effect of α-2,6-sialylation of the immunoglobulin moiety was excluded by observing that lymphocytes fail to bind to COS cells cotransfected with α-2,6ST and cell-surface CD22-encoding cDNA, and that binding is restored by subjecting the cotransfectants to *Au* sialidase cleavage (26). These results provide evidence that α-2,6-sialylation of CD22 may significantly alter its functional properties as both an adhesion and a costimulatory molecule.

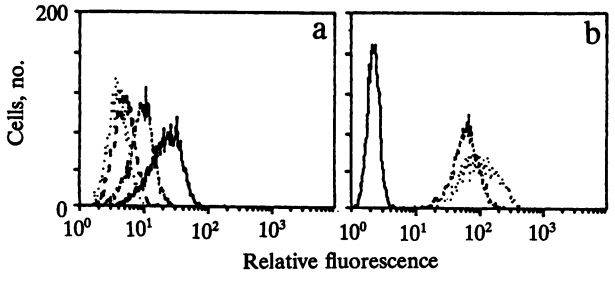

FIG. 5. Binding of α-2,3- and α-2,6-sialylated CD22Rg to Jurkat cells. Cells (10^6) were incubated with the following receptor globulins at 50 μg/ml and analyzed by flow cytometry: (*a*) CD22Rg (solid line), α-2,6-sCD22Rg (sparsely dotted line), α-2,6-sCD22Rg treated with *Au* sialidase (densely dotted line), and α-2,6-sCD22Rg treated with NDV sialidase (dashed line). (*b*) ——, CD44Rg; ----, CD22Rg; and •••••, α-2,3-sCD22Rg.

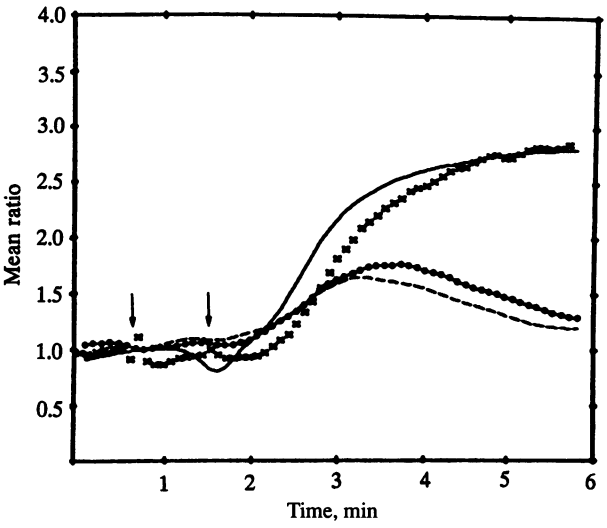

FIG. 6. Effect of sialic acid modification of CD22Rg on CD22Rg-induced calcium signals. ---, anti-CD3 mAb only; —, anti-CD3 mAb crosslinked to CD22Rg; •••, anti-CD3 mAb crosslinked to α-2,6-sCD22Rg; ▪▪▪, anti-CD3 mAb crosslinked to α-2,6-sCD22Rg treated with *Au* sialidase. Experiments were done under conditions identical to those described for Fig. 4.

DISCUSSION

Our work shows that CD22-dependent alteration of early T-cell activation requires CD22–CD45 interaction and that association between CD22 and CD45 can be inhibited by addition of sialic acid residues in α-2,6-linkage to the CD22 receptor. Interestingly, the stimulatory response of Jurkat cells to anti-CD3/CD22Rg crosslinking differs from the inhibitory effect of crosslinking anti-CD3 mAb and CD22Rg on the surface of normal T cells and the CEM T-cell line (4). These apparently contradictory data may reflect the different amounts of anti-CD3 used to trigger T cells in the two studies. However, these data may also suggest another regulatory mechanism of CD45 function. Because CD22 engagement of CD45 depends on α-2,6-sialylation of N-linked oligosaccharides associated with the CD45 protein core (5, 6) and because glycosylation of CD45 is cell-type specific (10), quantitative and qualitative differences in CD45-associated α-2,6-linked sialic acid among lymphocyte subsets might determine the fraction of CD45 molecules capable of engaging CD22 and the strength of CD22–CD45 association. In support of this view, a recent study has demonstrated that human leukocyte CD45 contains mainly bi-, tri-, and tetraantennary oligosaccharides that have exclusively α-2,6-linked sialic acid residues (27). Because CD22 binding to sialoglycoprotein ligands directly relates to the number of ligand oligosaccharide-associated α-2,6-linked sialic acid residues, variations in CD45 sialylation and sialic acid side-chain modification (28) may directly affect the strength of CD22–CD45 interaction. Stimulation or inhi-

bition of antigen-receptor signaling may, in turn, depend on the fraction of CD45 molecules that can bind CD22 and aggregate with the antigen-receptor complex and accessory molecules. The fraction of CD45 receptors that bind CD22 may be determined, in part, by expression of other CD22-binding sialoglycoproteins on T cells. Although engagement of these molecules by CD22Rg in the absence of wild-type CD45 does not modulate anti-CD3-induced T-cell activation, these molecules probably compete for CD22 binding with CD45 and thereby participate in regulating CD22–CD45 interaction.

That CD22-mediated enhancement of T-cell activation can be abrogated by α-2,6-sialylation of CD22 provides insight into the functional regulation of CD22. B cells possess a lineage-specific α-2,6ST promoter that is induced upon activation by antigen or mitogens, and activated B cells express a variety of α-2,6-sialylated cell-surface molecules that are absent from resting B cells (29). The adhesive and stimulatory properties of CD22 may, therefore, be regulated by the expression and activity of α-2,6ST. At low levels of α-2,6ST activity, as, for example, in resting B cells, CD22 ligand-binding epitopes may be free to interact with glycoproteins on adjacent cells. Upon B-cell activation and induction of α-2,6ST expression, CD22 ligand-binding sites may become occupied by sialic acid residues on CD22 itself or by those associated with adjacent molecules on the same cell surface. Hence, depending on the stage of B-cell activation, CD22 may participate in the regulation of signals generated by helper T cells as a result of T cell–B cell interaction or in the control of B-cell responses, where CD22 may facilitate aggregation of CD45 and surface IgM (30). High levels of B-cell α-2,6ST activity may potentially abrogate CD22-mediated interactions altogether. Sialylation in α-2,6-linkage may, therefore, provide a sensitive control mechanism of CD22 function. Although the physiologic implications of CD22–CD45 interaction remain to be elucidated, our observations provide evidence that engagement of CD45 by a natural ligand may be implicated in the regulation of antigen-receptor-complex signaling and proposes another mechanism for regulating receptor–ligand interaction that is intimately associated with expression of a specific glycosyltransferase.

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