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CD22, a B Cell-specific Immunoglobulin Superfamily Member, Is a Sialic Acid-binding Lectin*

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The B lymphocyte cell surface receptor CD22 is an adhesion molecule that can mediate binding to several leukocyte subsets. The first CD22 ligand to be identified was the receptor-linked phosphotyrosine phosphatase CD45, but several lines of evidence suggest that CD22 may interact with multiple counter receptors on adjacent lymphocytes. In the present work, we show that in addition to CD45, a soluble CD22-immunoglobulin fusion protein (CD22Rg) recognizes several other distinct lymphocyte sialoglycoproteins. CD22-mediated adhesion is dependent upon the presence of sialic acids on ligands. CD22Rg is observed to bind specifically to a 115-kDa sialoglycoprotein in COS cells transfected with an α-2,6-sialyltransferase cDNA, but not in COS cells transfected with unrelated cDNA clones, indicating that at least some CD22-mediated interactions require presentation of sialic acid in an α-2,6 linkage by CD22 ligands. In all cases, truncation of the side chain of sialic acids by mild periodate oxidation abolishes recognition by CD22Rg. Direct binding of CD22Rg to lymphoid cells also requires sialic acids and their side chains. Taken together, these observations indicate that CD22 is a sialic acid-binding lectin and may define a novel functional subset of immunoglobulin superfamily adhesion molecules.

Cell-cell adhesion plays a critical role in the normal function of the immune system, regulating lymphocyte response to antigenic challenge, recirculation to lymphoid tissues, and the extravasation of responder cells to sites of injury. Interactions among leukocytes and between leukocytes and endothelial cells are mediated by multiple adhesion molecules, most of which fall into three distinct classes, including immunoglobulin superfamily members, integrins, and selectins (Springer, 1990). Although no strict rules governing the types of interactions mediated by each family of molecules have been uncovered, experimental data so far suggest that most Ig-like molecules interact with other Ig-like receptors and integrins (Springer, 1990), whereas integrins bind specific peptide sequences of extracellular matrix proteins (Hemler, 1990; Hynes, 1987), in addition to certain Ig-like counterreceptors (Elices *et al.*, 1990; Hemler, 1990). Thus, both Ig-like adhesion molecules and integrins are thought to mediate primarily protein-protein interactions. However, an increasing number of leukocyte receptors are being found to display lectin activity by recognizing carbohydrate ligands on adjacent cells. So far, the best characterized leukocyte lectins include members of the selectin family, which recognize sialylated carbohydrates on adjacent cell surfaces (Walz *et al.*, 1990; Lowe *et al.*, 1990; Osborn, 1990; Moore *et al.*, 1992) and CD44H, the principal cell surface receptor for hyaluronate (Aruffo *et al.*, 1990). Each of these molecules contains a region within its extracellular domain which displays sequence homology to the C-type mammalian lectins (Stamenkovic *et al.*, 1989; Springer, 1990). Recently, we have reported that a B lymphocyte-specific Ig-like adhesion molecule, CD22, mediates cell-cell interaction by binding isoforms of the leukocyte-specific receptor-linked phosphotyrosine phosphatase, CD45 (Stamenkovic *et al.*, 1991; Aruffo *et al.*, 1992). Preliminary observations have suggested that CD22 may recognize specific carbohydrate residues expressed on CD45 (Stamenkovic *et al.*, 1991; Aruffo *et al.*, 1992), even though it does not possess a C-type lectin domain.

CD22 is a B cell lineage-restricted cell surface glycoprotein belonging to the immunoglobulin gene superfamily and is highly homologous to several adhesion molecules, including myelin-associated glycoprotein (MAG)[1] (Lai *et al.*, 1987), neural cell adhesion molecule (NCAM) (Cunningham *et al.*, 1987), and the vascular adhesion molecule V-CAM/InCAM110 (Osborn, 1990; Stamenkovic and Seed, 1990; Wilson *et al.*, 1991). Two independently expressed isoforms of CD22 have been identified. The smaller, 125-kDa isoform, designated CD22α, is composed of five Ig-like extracellular domains (Stamenkovic and Seed, 1990), whereas the larger, 135-kDa isoform, designated CD22β, is identical to CD22α with the exception of the presence of two additional Ig-like extracellular domains, inserted between Ig domains 2 and 3 of the smaller form (Wilson *et al.*, 1991; Stamenkovic *et al.*, 1991). CD22α, expressed in COS cells, mediates adhesion to monocytes and erythrocytes (Stamenkovic and Seed, 1990), whereas COS cells transfected with CD22β promote rosetting of B and T cells in addition to erythrocytes and monocytes (Stamenkovic *et al.*, 1991; Wilson *et al.*, 1991). The first three extracellular Ig-like domains of CD22β have been shown to contain sequences necessary for interaction with T and B cell ligands (Stamenkovic *et al.*, 1991).

We have reported previously that a recombinant soluble

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[1] The abbreviations used are: MAG, myelin-associated glycoprotein; NCAM, neural cell adhesion molecule; Rg, receptor-globulin; FBS, fetal bovine serum; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PHA, phytohemagglutinin.

form of CD22β (referred to as CD22Rg for receptor-globulin; Aruffo *et al.*, 1990), containing the first three Ig domains, interacts with several isoforms of CD45 on lymphoid cells (Stamenkovic *et al.*, 1991; Aruffo *et al.*, 1992). However, soluble CD22 has also been shown to specifically react with COS cells transfected with α-2,6-sialyltransferase (Stamenkovic *et al.*, 1991). The demonstration that α-2,6-sialyltransferase is located in the Golgi apparatus (Roth, 1987; Colley *et al.*, 1989; Munro, 1991) suggests that expression of the enzyme in COS cells alters the sialic acid composition of a variety of constitutive COS cell surface molecules, resulting in a novel adhesive phenotype that promotes interaction with CD22. In the present study we show that soluble CD22β recognizes multiple glycoprotein ligands on B and T cells, as well as a major 115-kDa sialoglycoprotein on α-2,6-sialyltransferase-transfected COS cells, and that CD22β-mediated adhesion is dependent upon the presence of sialic acid side chain residues on the ligands. These observations indicate a novel mechanism for the regulation of cell-cell adhesion by members of the Ig superfamily.

EXPERIMENTAL PROCEDURES

Materials—$Na^{125}I$, D-[6-^{3}H]glucosamine, L-[5,6-^{3}H]fucose, and D-[2-^{3}H]mannose were obtained from ICN (Costa Mesa, CA) and IODO-BEADS from Pierce Chemical Co. Sodium metaperiodate, sodium borohydride, EDTA, aprotinin, and DEAE-dextran were purchased from Sigma. Products for tissue culture, including Dulbecco's modified Eagle's medium, RPMI, and fetal bovine serum (FBS) were obtained from Irvine Scientific (Santa Ana, CA). Molecular biology reagents were from Boerhinger Mannheim, Promega (Madison, WI), and New England Biolabs (Beverly, MA). Monoclonal antibodies were purchased from Becton Dickinson (Mountainview, CA), and anti-CD45 mAb 9.4 was a kind gift from Alejandro Aruffo and Jeff Ledbetter (Bristol Myers-Squibb Pharmaceutical Research Institute, Seattle, WA). Secondary fluorescein-labeled antibodies were from Cappel, (Malvern, PA), and protein A-Sepharose beads were from Repligen (Boston, MA). Protein assay kits were obtained from Bio-Rad. Cell lines were obtained from American Type Culture Collection. Film for autoradiography was from Eastman Kodak, and enhancer Autofluor solution was from National Diagnostics (Manville, NJ).

Development and Production of Soluble Recombinant Fusion Proteins—Development of soluble CD22-immunoglobulin chimeras (CD22Rg) has been described previously (Stamenkovic *et al.*, 1991). Briefly, synthetic oligonucleotide primers complementary to the 5′ extremity of sequences encoding the CD22 secretory signal sequence and to the 3′ extremity of sequences encoding the third Ig-like domain of CD22 were used to direct amplification of the first three Ig-like domains. The 5′ and 3′ oligonucleotide primers were designed to include appropriate endonuclease restriction sites to facilitate directional incorporation into Ig expression vectors as described previously (Aruffo *et al.*, 1990; Stamenkovic *et al.*, 1991). Construction of CD5Rg (Aruffo *et al.*, 1990), CD40Rg (Noelle *et al.*, 1992), and Leu8Rg/L-selectinRg (Hollenbaugh *et al.*, 1992) were performed in a similar manner and have been described elsewhere. CD22-, CD40-, and Leu8-Ig chimeras were introduced into COS cells by the DEAE-dextran method (Aruffo and Seed, 1987), and 12 h after transfection the culture medium containing 10% FBS was removed and replaced with serum-free Dulbecco's modified Eagle's medium to eliminate contamination with serum-derived immunoglobulins. Cells were maintained in serum-free medium for 5–7 days and supplemented with 3–5 ml of fresh medium every 2 days. Five to seven days after transfection, supernatants were harvested and passed over protein A-Sepharose at a rate of 1 ml/min, the columns were washed three times with PBS, and bound Rg eluted with 0.1 M citric acid, pH 3.75. Eluates were immediately neutralized with 1 M Tris to a final pH of 7.5, dialyzed overnight against PBS, and the protein concentration determined using a Bio-Rad protein assay kit.

Surface Iodination and Immunoprecipitation—For surface iodination, 1×10^7 cells were washed in ice-cold PBS, resuspended in 1 ml of PBS, and incubated for 15 min with 1 mCi of NaI^{125} in the presence of IODO-BEADS. Labeled cells were washed four times in PBS and lysed in a buffer consisting of 0.5% Nonidet P-40, 0.02% NaN_3, and 20 μg/ml aprotinin. Nuclei and cytoskeletal matrix were removed by centrifugation for 10 min, and lysates were precleared with human IgG (10 μg/ml) and 25 μl of packed protein A-Sepharose beads. Precleared lysates were incubated with 50 μl of fresh protein A-Sepharose beads and 12–15 μg of Rg protein or 10 μg of 9.4 mAb for 4 h at 4 °C. Beads were washed four times in lysis buffer and precipitates eluted by boiling for 5 min in sample buffer in the presence of 2% 2-mercaptoethanol. Samples were subjected to sodium dodecyl sulfate-polyacrylamide gel (6–16%) electrophoresis, amd the gel were fixed and exposed to x-ray film for 12–48 h at −80 °C.

Mild Periodate Oxidation and Borohydride Reduction—A fresh stock of 100 mM sodium metaperiodate in PBS, pH 7.2, was prepared for each experiment. Phytohemagglutinin (PHA) blasts, T and B cell lines were washed in ice-cold PBS, resuspended in PBS with 2 mM sodium periodate at 1×10^6 cells/ml, and incubated on ice in the dark for 15 min (Van Lenten and Ashwell, 1971; Gahmberg and Andersson, 1977). Cells were washed in ice-cold PBS and were stained with either Rg molecules or mAb, followed by a secondary fluorescein-labeled antibody, and subjected to analysis on a FACS analyzer (Becton-Dickinson), or radiolabeled as described above and lysed for immunoprecipitation.

Following periodate oxidation, some cells were subjected to treatment by sodium borohydride to reduce the aldehydes generated by periodate. A fresh stock of 2 M sodium borohydride was prepared for each experiment. After two washes in cold PBS, periodate-treated cells were resuspended in PBS with 20 mM sodium borohydride in an 18 °C water bath for 20 min, washed in cold PBS, and subjected to immunoprecipitation studies as above. Control cells were subjected to the same incubation conditions without sodium metaperiodate or borohydride addition and with borohydride treatment alone.

Cell Surface Staining—Cells were washed in cold PBS, resuspended in PBS, 0.02% azide, and incubated with 20 μg/ml of CD22Rg or Leu8Rg for 1 h on ice; B lymphocyte lines were preincubated with 10 μg/ml rat IgG in an effort to block Fc receptor binding of Rg molecules. Cells were washed in PBS, resuspended in PBS, 0.02% azide and 5 μg/ml fluorescein-conjugated, affinity-purified goat anti-mouse antibody (Cappel) for 30 min on ice, washed, fixed in 4% formaldehyde, and analyzed on a FACS analyzer.

Metabolic Labeling and Cell Culture—COS cells were transfected with α-2,6-sialyltransferase cDNA (Stamenkovic and Seed, 1990) with the DEAE-dextran method as described (Aruffo and Seed, 1987). Twelve to twenty-four h later, transfected cells were detached with trypsin and replated onto fresh plates in RPMI, 10% FBS. To label the oligosaccharides of CD22 ligands metabolically, transfected COS cells and 3.2×10^6 cells from the lymphoid cell lines CEM and Raji as well as PHA blasts were incubated with 100 μCi each of [6-^{3}H] glucosamine hydrochloride, [2-^{3}H]mannose, or [5,6-^{3}H]fucose in 8 ml of RPMI, 10% FBS for 48 h. PHA blasts were generated by isolating peripheral blood mononuclear cells on a Ficoll gradient and incubating 3.2×10^6 of the Ficoll-separated cells in RPMI, 10% FBS supplemented with 1 μg/ml PHA. Radiolabeling of PHA-stimulated T cells was begun 24 h following stimulation and allowed to proceed for 48 h. After incubation cells were washed three times in PBS; COS cells were detached with EDTA. Cells were lysed and lysates subjected to precipitation with CD22Rg or control L-selectinRg molecules as above. Precipitates were subjected to sodium dodecyl sulfate-polyacrylamide gel (8%) electrophoresis under reducing conditions, the gels fixed in 30% methanol, 7% acetic acid, enhanced in Autofluor (National Diagnostics) in the presence of 10% glycerol, dried, and exposed to x-ray film.

To determine the relative incorporation of monosaccharide labels into B and T cell CD22 ligands with respect to total cellular glycoproteins, a fraction of metabolically labeled total cell lysate from Raji cells and PHA blasts and an equal fraction of the corresponding CD22Rg immunoprecipitates were acetone precipitated at an acetone:lysate ratio of 4:1 at −80 °C overnight, and the precipitate was resuspended in 0.1% Nonidet P-40. The amount of radioactivity in each fraction was determined in a β-scintillation counter, and the CD22Rg ligand incorporation was expressed as a percentage of the total incorporation.

RESULTS

Identification of Multiple B Cell and T Cell Surface Ligands for CD22β—To facilitate the identification of CD22 ligands and subsequent studies on receptor-ligand interactions, a CD22-immunoglobulin fusion protein, termed CD22Rg, was developed (Stamenkovic *et al.*, 1991) comprising the first three CD22 Ig domains, linked to human IgG Fc regions (Fig. 1).

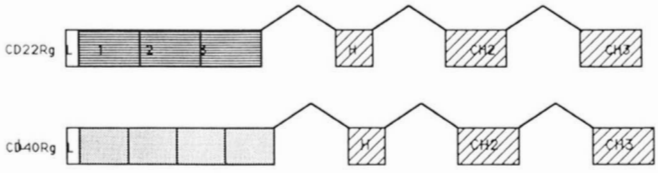

FIG. 1. **CD22 and CD40 receptor-globulin constructs.** CD22 Ig-like domains and CD40 tandem repeats are *shaded*, and CD22 domains are *numbered*. Human IgG1 exons are *boxed*, and introns are denoted by *connecting lines*. *L*, leader sequence; *H*, hinge region; *CH2* and *CH3*, constant region exons 2 and 3.

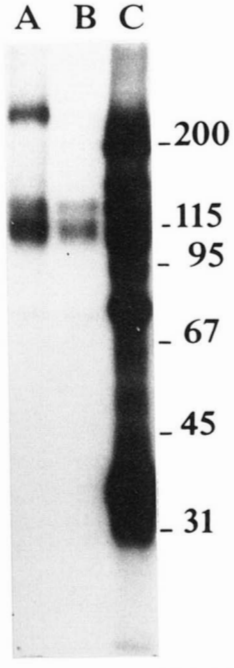

FIG. 2. **Specific precipitation of CD22 ligands from T and B cell lines using CD22Rg.** T and B cell lines were surface radiolabeled with ^{125}I, lysed, and lysates immunoprecipitated with CD22Rg. CD22Rg immunoprecipitates of lysates derived from T cell line CEM (*lane A*), T cell line Molt4 (*lane B*), Burkitt lymphoma cell line Daudi (*lane C*). Molecular mass markers (*kd*) are indicated.

CD22Rg was shown to bind B and T cells as assessed by indirect immunofluorescence (Stamenkovic *et al.*, 1991). The observation that CD22Rg binds several CD45 isoforms (Aruffo *et al.*, 1992) could explain its reactivity with both T and B cells. However, some lymphoid cell lines that express CD45 weakly or not at all were observed to react with CD22Rg,[2] suggesting the existence of CD22 ligands other than CD45 and raising the possibility that CD45-positive lymphocytes might express additional CD22 ligands on their surface. In an attempt to identify novel putative CD22β B and T cell ligands, CD22Rg was used to precipitate glycoproteins from ^{125}I-labeled PHA-stimulated peripheral blood T cells and from a variety of B and T cell lines. Normal T cells were PHA stimulated prior to labeling because previous observations indicated that stimulated T cells display greater CD22Rg reactivity than resting counterparts (Stamenkovic *et al.*, 1991; Aruffo *et al.*, 1992).

Precipitation with protein A-Sepharose-bound CD22Rg showed that CD22Rg reacts with several cell surface proteins on T and B cell lines (Fig. 2). Immunoprecipitates from T cell lines revealed differences in the types and relative abundance of immunoprecipitated molecules. A subclone of the T cell leukemia Jurkat gave two major bands of 130 and 115 kDa and a faint 220-kDa species consistent with a CD45 isoform (data not shown). CD22Rg precipitates of lysates from two other T cell lines, Molt4 and CEM, gave similar patterns, with prominent 115- and 130-kDa polypeptides similar to those from Jurkat lysates, as well as a 220–240-kDa species in the CEM lysate (Fig. 2). Molt4-derived precipitates disclosed very faint 200- and 180-kDa bands that correlated with low expression of CD45 isoforms (data not shown). Precipitates from PHA blast-derived lysates showed a more complex pattern, including, in addition to the 115- and 130-kDa species, major bands of 200 and 150/160 kDa, as well as several minor species (see Fig. 6). All of the observed bands appeared to be specific for CD22Rg activity, as CD40Rg failed to precipitate any of the proteins reactive with CD22Rg (Fig. 2, data not shown).

CD22Rg precipitated several polypeptides from B cell lines. Generally, B cell lines appeared to express a greater number of CD22-binding glycoproteins than T cell lines. CD22Rg precipitates of radiolabeled lysates from the Burkitt lymphoma Daudi, for example, were characterized by the presence of multiple bands, including species of 220, 200, 160, 115, 90, 70, and 32 kDa (Figs. 2 and 6). Lysates from the Burkitt lymphoma Raji, on the other hand, contained a smaller number of CD22Rg-binding polypeptides (Fig. 3) and lacked the 32-kDa species observed in precipitates from Daudi lysates (data not shown). However, the 220- and 200-kDa species were precipitated from both lysates (Figs. 2 and 3), as were the 115- and 130-kDa glycoproteins which appear to be expressed by B and T cells alike (Figs. 2, 3, and 6). The 220-kDa band was observed to co-migrate with the Raji cell CD45 isoform immunoprecipitated with the anti-CD45 mAb 9.4 (Ledbetter *et al.*, 1985). Direct interaction between CD22 and CD45 was shown previously (Aruffo *et al.*, 1992), and the present comparison was included to distinguish the other ligands from CD45. It is noteworthy that similar amounts of the CD45 isoform were precipitated by both antibodies and CD22Rg.

B and T Cell Ligands of CD22β Are Cell Surface Glycoproteins—To elucidate some of the structural characteristics of CD22 ligand-associated carbohydrates, the T cell line CEM and Burkitt lymphoma Raji cell line as well as PHA blasts were metabolically radiolabeled with [6-^{3}H]glucosamine, [5,6-^{3}H]fucose, and [2-^{3}H]mannose for 48 h, lysed, and lysates precipitated with CD22Rg and L-selectinRg controls. CD22-specific ligands from both cell lines as well as from PHA blasts incorporated glucosamine and fucose (Fig. 4 and data not shown) and, more weakly, mannose. The major 200–220-kDa species, which was similar in both cell types, showed

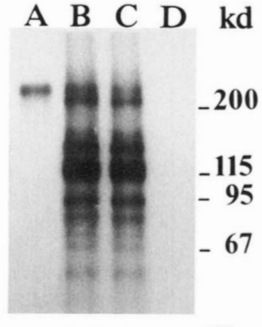

FIG. 3. **Specific precipitation of ^{125}I-surface-labeled Raji cell lysates with anti-CD45 mAb and CD22Rg.** Specific precipitation was performed with anti-CD45 mAb 9.4 (*lane A*), CD22Rg (*lane B*), CD22Rg in the presence of 5 mM EDTA (*lane C*), CD22Rg following treatment of Raji cells with neuraminidase (*lane D*).

[2] D. Sgroi and I. Stamenkovic, unpublished data.

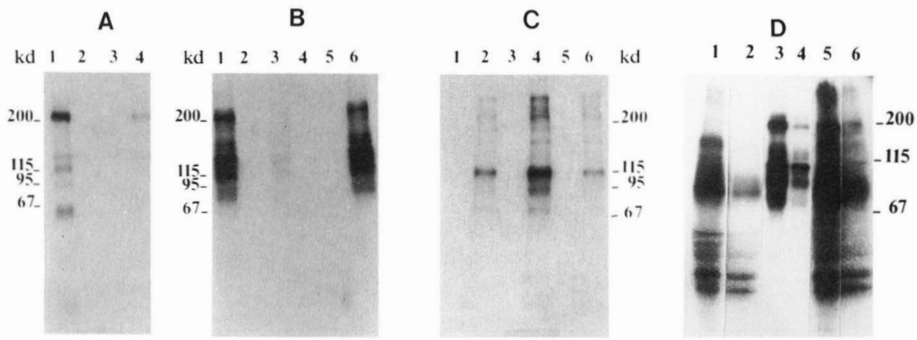

FIG. 4. **Specific precipitates of T cell, B cell, and α-2,6-sialyltransferase-transfected COS cell lysates from cells labeled with [^{3}H]glucosamine, [^{3}H]fucose, and [^{3}H]mannose, using CD22Rg and Leu8Rg.** *Panel A*, precipitates from the T cell line CEM labeled with [^{3}H]fucose (*lanes 1* and *3*) and [^{3}H]mannose (*lanes 2* and *4*). Immunoprecipitates were obtained with CD22Rg (*lanes 1* and *4*) and Leu8Rg (*lanes 2* and *3*). *Panel B*, immunoprecipitation of Raji cell lysates radiolabeled with [^{3}H]glucosamine (*lanes 1* and *2*), [^{3}H]mannose (*lanes 3* and *4*), and [^{3}H]fucose (*lanes 5* and *6*). Lysates were immunoprecipitated with CD22Rg (*lanes 1*, *3*, and *6*) and Leu8Rg (*lanes 2*, *4*, and *5*). *Panel C*, immunoprecipitates from α-2,6-sialyltransferase-transfected COS cell lysates radiolabeled with [^{3}H]fucose (*lanes 1* and *2*), [^{3}H]glucosamine (*lanes 3* and *4*), and [^{3}H]mannose (*lanes 5* and *6*). Immunoprecipitates were obtained with CD22Rg (*lanes 2*, *4*, and *6*) and Leu8Rg (*lanes 1*, *3*, and *5*). *Panel D*, comparison of [^{3}H]glucosamine- (*lanes 1*, *3*, and *5*) and [^{3}H]fucose- (*lanes 2*, *4*, and *6*) radiolabeled acetone precipitates of Raji cell lysates before and after precipitation by CD22Rg. *Lanes 1* and *2* are acetone precipitates after CD22Rg precipitation; *lanes 3* and *4*, CD22Rg precipitates; *lanes 5* and *6*, acetone precipitates prior to CD22Rg precipitation. Molecular mass markers (*kd*) are indicated.

TABLE I

Incorporation of [^{3}H]glucosamine, [^{3}H]fucose, and [^{3}H]mannose into CD22Rg-binding molecules

Radiolabeled Raji and CEM cell lysates were acetone precipitated or CD22Rg immunoprecipitated, and radiolabel incorporated into corresponding precipitates was compared. The counts presented correspond to 1/50 of acetone-precipitated label and 1/25 of CD22Rg-associated label.

Radioactivity incorporated from	Raji cells			CEM cells		
	Acetone precipitable	CD22Rg bound	% CD22Rg bound	Acetone precipitable	CD22Rg bound	% CD22Rg bound
[^{3}H]Glucosamine	206,158	8,142	1.9	46,736	1,202	1.3
[^{3}H]Fucose	18,622	283	0.76	5,381	73	0.67
[^{3}H]Mannose	50,802	4,605	4.5	22,142	355	0.8

incorporation of all three monosaccharides to varying degrees. Two molecules of 115- and 130-kDa were also prominent in CD22Rg precipitates from all three lysates and correspond to bands observed in precipitates of surface-iodinated cells (Fig. 4). Differences in the CD22Rg precipitation patterns between the two cell lines were observed, in that several species precipitated from Raji cell lysates were absent in CEM lysates. These patterns correspond to those observed in immunoprecipitates from lysates of ^{125}I surface-labeled cells. However, some of the low molecular weight species immunoprecipitated from surface-labeled lysates failed to appear in immunoprecipitates of lysates derived from metabolically labeled cells. These species could have low turnover rates, resulting in poor metabolic labeling.

To estimate the fraction of total cellular glycoprotein-incorporated monosaccharide associated with CD22-binding glycoproteins, radioactivity incorporated in specific CD22Rg precipitates was measured and expressed as a percentage of total radioactivity released by corresponding acetone-precipitated lysates (Table I). CD22Rg precipitates of Raji and CEM cell lysates were found to contain, respectively, 1.9 and 1.3% of total acetone precipitate-incorporated [^{3}H]glucosamine, 0.75 and 0.67% of total acetone-precipitable [^{3}H]mannose, and 4.5 and 0.8% of total acetone precipitate-incorporated [^{3}H]fucose. The observation that CD22Rg ligand-associated carbohydrates constitute a low percentage of total glycoprotein-associated carbohydrates supports the notion that CD22Rg recognizes a small number of specific ligands. Comparison of [^{3}H]fucose- and [^{3}H]glucosamine-radiolabeled, acetone-precipitated total Raji cell lysates to acetone precipitated lysates following precipitation by CD22Rg shows depletion of bands corresponding to specific CD22Rg-binding molecules (Fig. 4).

CD22 Interaction with Ligands Depends on the Presence of Sialic Acid on Ligands but Does Not Require Divalent Cations—We had shown previously that pretreatment of T and B cells with sialidase abrogates CD22β-mediated lymphocyte rosetting (Stamenkovic *et al.*, 1991; Aruffo *et al.*, 1992). To determine whether interaction with all of the ligands is sialic acid-dependent, Raji cells and PHA blasts were subjected to treatment with sialidase, ^{125}I radiolabeled, lysed, and the lysates precipitated with CD22Rg. Sialidase treatment completely abrogated CD22Rg-ligand interaction with both Raji cell (Fig. 3) and PHA blast (data not shown) ligands. This observation confirms and extends the previous findings since it directly demonstrates that CD22Rg binding to each of its cell surface glycoprotein ligands requires the presence of ligand-associated sialic acid. Adhesion mediated by several mammalian lectins, most notably members of the selectin family, requires the presence of divalent cations. To test whether this holds true for CD22, radiolabeled Raji cell and PHA lysates were immunoprecipitated with CD22Rg in the presence of EDTA (Fig. 3 and data not shown). However, no modification of the immunoprecipitates was observed with respect to those obtained in the absence of EDTA, indicating that unlike the selectins, CD22 does not require divalent cations to bind to its ligands.

Sialic Acid Side Chains of B and T Cell Ligands Are Required for CD22β-mediated Adhesion—Since sialidase treatment of lymphocytes completely abolished CD22-ligand binding, we next tried to determine whether CD22Rg interaction with ligands requires specific aspects of sialic acid structure. To test whether T and B cell ligands require the presence of sialic acid side chains for interaction with CD22β, T cell blasts, and T and B cell lines were briefly subjected to mild sodium metaperiodate oxidation prior to immunofluorescence staining with CD22Rg. Under these mild conditions, sodium metaperiodate oxidation selectively cleaves the polyhydroxylated tail of sialic acid, resulting in the 8-carbon analogue (5-acetamido-3,5-dideoxy-D-galactosyloctulosonic acid) or the 7-carbon analogue (5-acetamido-3,5-dideoxy-D-galactosylheptulosonic acid) with a free aldehyde group at the cleavage site (Van Lenten and Ashwell, 1971; Schauer, 1982). Under these conditions, the main ring structure of the sialic acid and the underlying oligosaccharide structures remain unchanged. In all cases, CD22Rg reactivity was abolished in mild periodate-treated B and T cell lines in immunofluorescence FACS assays (Fig. 5). This effect was confirmed by performing direct precipitation of lysates derived from periodate-treated T and B cells with CD22Rg. Again, mild periodate oxidation completely abrogates CD22Rg interaction with all T and B cell ligands (Fig. 6).

Mild periodate treatment oxidizes and cleaves the sialic acid side chain, leaving behind a truncated side chain with a terminal aldehyde group instead of a hydroxyl group (Van Lenten and Ashwell, 1971; Schauer, 1982). Thus, periodate treatment of cells expressing CD22 ligands may abrogate CD22Rg binding by at least two mechanisms. One possibility is that the cleavage of the terminal carbon moeity results in a loss of components responsible for recognition. This would support the notion that ligand-associated sialic acid side chains play an important role in CD22-mediated adhesion. A second possibility is that the newly formed aldehyde group interferes with binding; it is conceivable that the bond angle change between the terminal carbon and oxygen atom might result in a conformational change that interferes with binding or that the aldehydes might react nonspecifically with other adjacent compounds. To determine whether the aldehyde group is responsible for the loss of binding, mild periodate-treated T and B cells were briefly incubated with sodium borohydride, which reduces the oxidized side chain, thus restoring a primary hydroxyl group of sialic acid but leaving the side chain truncated (Van Lenten and Ashwell, 1971; Schauer, 1982). As determined by both immunofluorescence staining (data not shown) and immunoprecipitation (Fig. 7), sodium borohydride reduction of mild periodate-treated cells did not restore CD22Rg binding to PHA blasts or Raji cells (Fig. 7), and borohydride alone had no effect on binding (data not shown). These results suggest that periodate-induced inhibition of binding was not simply the result of generating an aldehyde group but rather that the presence of intact sialic acid side chains is critical for CD22 interaction with its ligands.

α-2,6-Sialyltransferase Promotes Ligand Recognition by CD22—Since sialic acid linkage is determined by specific

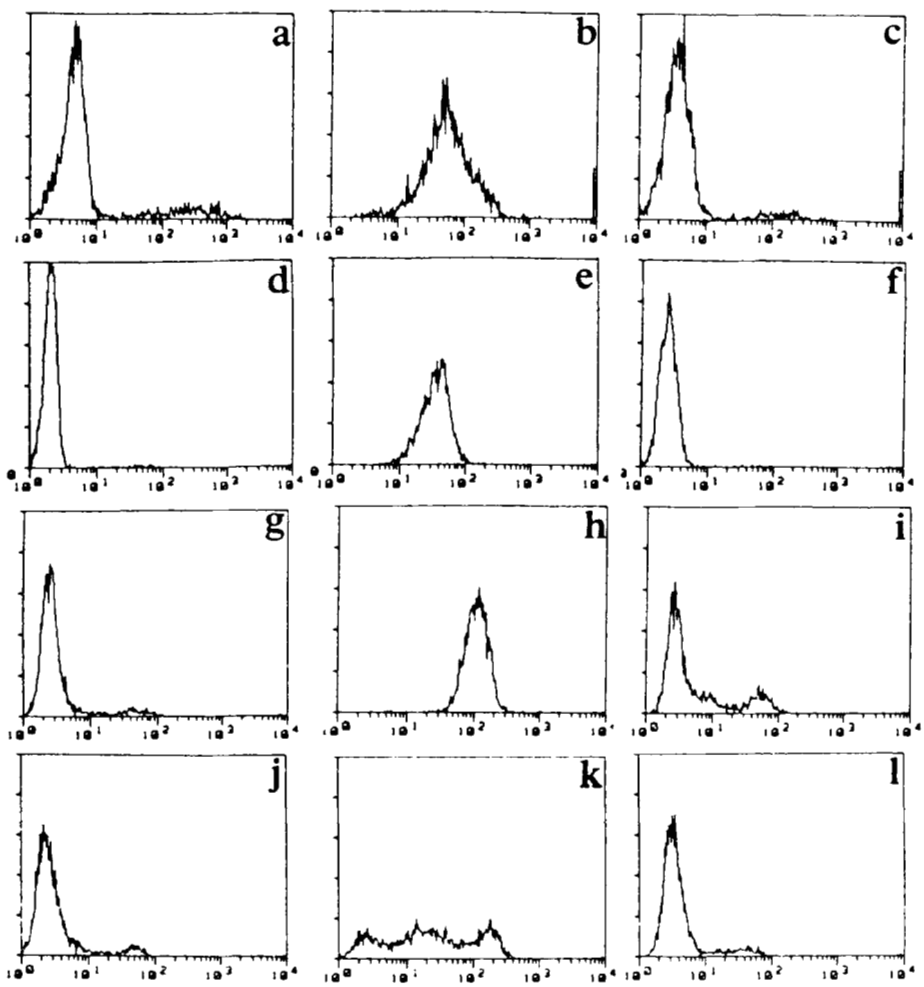

FIG. 5. **Reactivity of CD22Rg with lymphoid cells lines prior to and following periodate treatment as assessed by indirect immunofluorescence and FACS analysis.** Cell lines tested were PHA blasts (*panels a–c*), T cell leukemia CEM (*panels d–f*), pre-B cell leukemia Nalm6 (*panels g–i*), and Burkitt lymphoma Raji (*panels j–l*). Cell lines were tested for reactivity with CD40Rg control (*panels a, d, g,* and *j*), CD22Rg (*panels b, e, h,* and *k*), and CD22Rg following periodate treatment (*panels c, f, i,* and *l*).

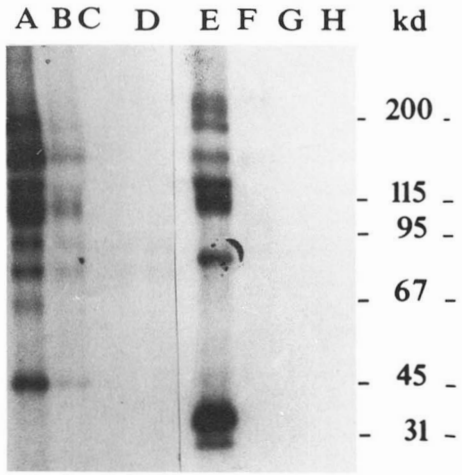

FIG. 6. **Mild periodate treatment of T and B cells.** Immunoprecipitates from ^{125}I-surface-labeled PHA blasts (*lanes A–D*) and the Burkitt lymphoma Daudi (*lanes E–H*) are shown. Immunoprecipitates from intact cell lysates (*lanes A* and *E*) and lysates from periodate-treated cells (*lanes B* and *F*) obtained using CD22Rg and intact cell lysates (*lanes C* and *G*) and lysates from cells treated with periodate (*lanes D* and *H*) obtained using CD40Rg are shown.

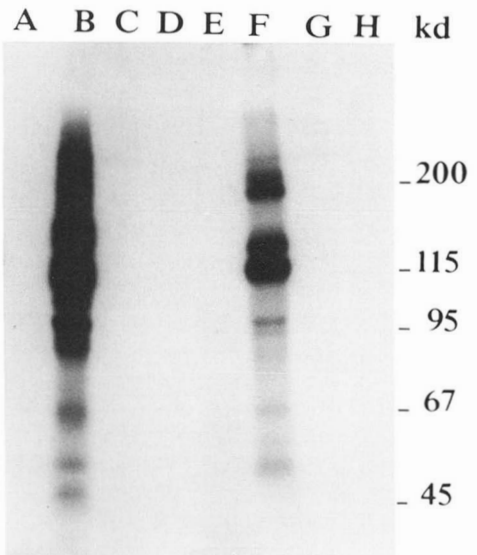

FIG. 7. **Mild periodate and borohydride treatment of ^{125}I-surface-labeled B and T cells.** Precipitates from PHA blast lysates (*lanes A–D*) and Raji cell lysates (*lanes E–H*) were obtained using CD40Rg (*lanes A* and *E*) and CD22Rg (*lanes B–D* and *F–H*). Lysates were derived from intact cells (*lanes A, B, E,* and *F*), cells treated with mild periodate (*lanes C* and *G*), and cells treated with mild periodate followed by borohydride (*lanes D* and *H*).

sialyltransferases, we tested whether an α-2,6 linkage might confer ligand affinity for CD22. We had shown previously that transfection of α-2,6-sialyltransferase into COS cells confers reactivity with CD22Rg as assessed by indirect immunofluorescence (Stamenkovic *et al.*, 1991). However, the issue had not been resolved as to whether CD22Rg recognizes one or several α-2,6-sialylated glycoproteins on COS cells or whether CD22Rg binding might be confined to oligosaccharides unassociated with polypeptides (*e.g.* cell surface glycolipids). COS cells transfected with α-2,6-sialyltransferase were ^{125}I labeled and radiolabeled lysates immunoprecipitated using CD22Rg and L-selectinRg controls. A major 115-kDa band was shown to be immunoprecipitated from lysates derived from α-2,6-sialyltransferase-transfected but not from mock transfected COS cells (Fig. 8). An L-selectinRg control failed to immunoprecipitate a similar polypeptide (Fig. 8). These observations suggest that CD22Rg recognizes at least one sialoglycoprotein containing sialic acid in α-2,6 linkage on the surface of COS cells. However, they do not exclude the possibility that additional molecular species, such as gangliosides substituted in α-2,6-linked sialic acid, might also be recognized in the intact cell.

To compare the COS cell α-2,6-sialyltransferase-dependent ligand to the 115-kDa ligand expressed in B and T cells, [6-^{3}H]glucosamine-, L-[5,6-^{3}H]fucose-, and D-[2-^{3}H]mannose-labeled COS cell lysates were immunoprecipitated with CD22Rg or Leu-8Rg (L-selectinRg) controls (Fig. 4). The CD22 ligand was found to incorporate all three monosaccharides. It is attractive to speculate that the 115-kDa COS cell ligand might be the simian homologue of the human lymphocyte 115-kDa glycoprotein which can bind CD22Rg only when appropriately substituted in α-2,6-linked sialic acid. Elucidation of the identity of these molecules will therefore be of considerable interest.

DISCUSSION

The present work demonstrates that CD22 behaves as a sialic acid-binding lectin, recognizing several lymphoid sialoglycoproteins. In general, sialic acids tend to repel cell-cell interactions by virtue of negative charge. However, certain mammalian sialic acid-binding lectins, which constitute a small group of structurally diverse molecules, appear to play a role in adhesion (Varki, 1992a, 1992b). The best characterized sialic acid-binding lectins belong to the selectin family of adhesion molecules composed of three highly related cell surface glycoproteins whose extracellular region features a C-type-like lectin domain, an epidermal growth factor-like domain, and a variable number of complement receptor domains (Bevilacqua *et al.*, 1989; Lasky *et al.*, 1989; Siegelman *et al.*, 1989; Johnston *et al.*, 1989). Interaction between selectins and their carbohydrate ligands plays a key role in the regulation of lymphocyte recirculation (Osborn, 1990) and leukocyte and platelet adhesion to the endothelium at sites of inflammation (Bevilacqua *et al.*, 1987). Additional sialic acid-binding lectins include the recently characterized sialoadhesin (Crocker *et al.*, 1990, 1991; van den Berg *et al.*, 1992), which is thought to

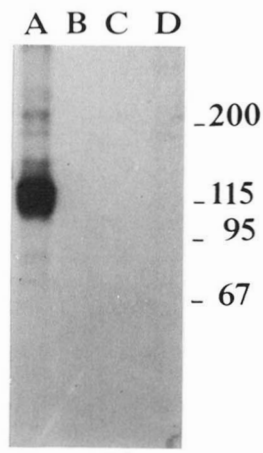

FIG. 8. **Immunoprecipitation of lysates from ^{125}I-radiolabeled α-2,6-sialyltransferase-transfected COS cells.** Immunoprecipitates were obtained using CD22Rg (*lanes A* and *B*) and L-selectinRg (*lanes C* and *D*). Lysates were from α-2,6-sialyltransferase cDNA-transfected COS cells (*lanes A* and *C*) and COS cells transfected with unrelated cDNA clones (*lanes B* and *D*).

promote macrophage-lymphocyte interactions (Crocker *et al.*, 1990; van den Berg *et al.*, 1992); the H protein of the alternate complement pathway (Meri and Pangburn, 1990); neural cell-associated ganglioside-binding protein (Tiemeyer *et al.*, 1990); and human placental lectin, believed to be present in the extracellular matrix (Ahmed and Gabius, 1989). CD22 shows no homology to the selectins. Because the primary structure of several of the other molecules has not been elucidated, their relatedness to CD22 cannot be determined. It is noteworthy, however, that CD22 displays functional similarity to sialoadhesin. Similar to placental lectin, but in contrast to the selectin family, sialoadhesin- and CD22-ligand interactions are independent of divalent cations. Both sialoadhesin and CD22 recognize ligands on erythrocytes and B and T cells, and the interaction is completely abrogated by sialidase treatment of ligand-bearing cells. However, CD22 is B cell-specific and selectively recognizes ligands expressing sialic acid in α-2,6 linkage (see Powell *et al.*, 1993), whereas sialoadhesin is expressed by macrophages and recognizes α2–3-sialic acid linkages in the Siaα-2,3Galβ1;-3GalNAc sequence, of *O*-linked structures and gangliosides (van den Berg *et al.*, 1992).

CD22 is closely related to several Ig-like molecules and particularly to the neural adhesion molecules MAG and NCAM. MAG and NCAM have been implicated in homotypic adhesion (Hoffman and Edelman, 1983; Lai *et al.*, 1987), and both molecules contain the HNK1 carbohydrate epitope, which appears to participate directly in adhesion events (Kruse *et al.*, 1984; Kunemund *et al.*, 1988). However, HNK1, which is a glycolipid (Ariga *et al.*, 1987), does not contain sialic acid (Stults *et al.*, 1989), suggesting an adhesion mechanism distinct from that observed with CD22. NCAM is a sialoglycoprotein (Hoffman and Edelman, 1983; Hemperly *et al.*, 1986), but the level of its polysialic acid content is thought to regulate the rate of homotypic adhesion negatively (Hoffman and Edelman, 1983; Rutishauser, 1989). Moreover, the sialic acid-rich and the ligand-binding domains of NCAM are distinct, and sialic acid does not appear to participate directly in interactions with ligand (Cunningham *et al.*, 1983). Most other Ig-like adhesion molecules interact with integrins or other Ig family members (Springer, 1990), but so far, none has been shown to be sialic acid-binding lectins.

The sialic acid binding property of CD22 appears to be unique among Ig-like receptors and may define a novel functional class of Ig adhesion molecules. Particularly relevant is the observation that ligand-associated sialic acid side chains are required for CD22 binding. Sialic acids are a diverse group of acidic sugars, whose only common denominator is a nine-carbon backbone and an α linkage, and there are numerous instances in which specific types of sialic acids mediate specific binding phenomena (Varki, 1992a, 1992b). For example, several paramyxoviruses viruses bind only to sialyl-oligosaccharide chains having α2–3 linkages and not to corresponding α-2,6 groups (Cahan and Paulson, 1980), whereas influenza C binds specifically to 9-*O*-acetylated sialic acid residues (Rogers *et al.*, 1986; Muchmore and Varki, 1987). Several other types of viruses preferentially bind receptors with α-2,8-linked sialic acid (Markwell and Paulson, 1980). The finding that α-2,6-sialyltransferase can confer CD22 affinity to at least one constitutive COS cell polypeptide supports the notion that CD22 might preferentially bind sialyl-oligosaccharide chains that have α-2,6 linkages. This prediction has been confirmed and extended by studying *N*-linked oligosaccharides from the cell types recognized by CD22 (Powell *et al.*, 1993). However, α-2,6-sialyltransferase transfers sialic acid residues to terminal carbohydrates on a vast number of glycoproteins, and the observation that α-2,6-sialyltransferase expression in COS cells promotes CD22-reactivity of a single ^{125}I-labeled polypeptide suggests that CD22 does not bind all sialoglycoproteins bearing α-2,6-linked sialic acid indiscriminately. Rather, the number and spacing of the α-2,6-linked sialic acid residues on multiple antennae of *N*-linked oligosaccharides appear to be important (Powell *et al.*, 1993). It is also possible that the protein structure of the ligand presenting the α-2,6-linked sialic acid, the underlying monosaccharide, or sialic acid substitutions may play a critical role in regulating binding to CD22. It would also seem reasonable to predict that the binding specificities of CD22 could be determined and modulated by changes in the type, quantity, and linkage of sialic acid in the natural ligands.

The abolition of binding by mild periodate oxidation with or without subsequent reduction suggests that the side chain(s) of sialic acid(s) on the ligands is(are) required for binding. This is confirmed in the accompanying paper (Powell *et al.*, 1993), which shows that mild periodate oxidation of sialic acids on *N*-linked oligosaccharides from the ligands abolishes their binding to CD22. This result is in striking contrast to the selectin family of cell adhesion molecules, which also recognize sialic acid bearing ligands. The side chain of sialic acids are not required for binding by E-selectin (Tyrrell *et al.*, 1991), or L-selectin (Norgard *et al.*, 1993). In the latter case, recognition is actually enhanced after oxidation, apparently because of Schiff base formation between the aldehydes on the side chains and amino groups on the receptor.

Recent evidence has shown that one group of CD22 ligands are CD45 isoforms and that binding to a single isoform, CD45RO, may vary among different CD45RO-expressing cells (Aruffo *et al.*, 1992). Since cell type-specific glycosylation is one mechanism of generating diversity among CD45 isoforms, the observed differences in CD22 binding may reflect ways in which cells regulate adhesion. Moreover, the extracellular domain of CD45 may serve as a polypeptide backbone or scaffolding that provides a means to present carbohydrate molecules to ligands thereby helping to regulate adhesion and consequent cytoplasmic domain-associated phosphotyrosine phosphatase activity. Several examples of biologically relevant interactions resulting from carbohydrate presentation by ligands to specific receptors are known. The first is illustrated by the mouse egg glycoprotein ZP3 which serves as a ligand for a sperm-binding protein in fertilization. The polypeptide backbone of ZP3 presents *O*-linked oligosaccharides that constitute the sperm binding determinants (Florman and Wassarman, 1985; Wassarman, 1990). The second is provided by the recently identified molecule sgp50 (Lasky *et al.*, 1992), a highly glycosylated membrane-associated protein that presents specific carbohydrates to L-selectin expressed on leukocytes and is thought to participate in the regulation of leukocyte trafficking. In addition, interaction between a sialylated 120-kDa myeloid cell glycoprotein and P-selectin, which is thought to facilitate myeloid cell adhesion to platelets and endothelial cells, is shown to recognize selectively the appropriate presentation of sialic acids by the ligand to P-selectin (Moore *et al.*, 1992). The observed CD22-binding glycoproteins might each consist of a protein core capable of presenting sialic acid groups to CD22 in a manner that promotes adhesion, and possibly, signal transduction. We have shown recently that interaction between CD22Rg and its T cell ligands induces signals that block CD3-driven T cell activation, closely resembling the effect of cross-linking CD3 and CD45 (Aruffo *et al.*, 1992). Whether this effect is solely because of interaction with CD45 or whether other ligands are implicated remains to be determined. Characterization of

the structure of the newly identified CD22 ligands and definition of the specificities of the presented sialyloligosaccharides will be critical to elucidate fully what appears to be a novel mechanism of lymphocyte adhesion.

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