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Cutting Edge: CD43 Functions as a T Cell Counterreceptor for the Macrophage Adhesion Receptor Sialoadhesin (Siglec-1)

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Sialoadhesin (Sn, also called Siglec-1)2 is a macrophage-restricted sialic acid-binding receptor that mediates interactions with hemopoietic cells, including lymphocytes. In this study, we identify sialoadhesin counterreceptors on T lymphocytes. Several major glycoproteins (85, 130, 240 kDa) were precipitated by sialoadhesin-Fc fusion proteins from a murine T cell line (TK-1). Binding of sialoadhesin to these glycoproteins was sialic acid dependent and was abolished by mutation of a critical residue (R97A) of the sialic acid binding site in the membrane distal V-set Ig domain of sialoadhesin. The 130- and 240-kDa sialoadhesin-binding glycoproteins were identified as the sialomucins CD43 and P-selectin glycoprotein ligand 1 (CD162), respectively. CD43 expressed in COS cells supported increased binding to immobilized sialoadhesin. Finally, sialoadhesin bound different glycoforms of CD43 expressed in Chinese hamster ovary cells, including unbranched (core 1) and branched (core 2) O-linked glycans, that are normally found on CD43 in resting and activated T cells, respectively. These results identify CD43 as a T cell counterreceptor for sialoadhesin and suggest that in addition to its anti-adhesive role CD43 may promote cell-cell interactions.

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2 Abbreviations used in this paper: Sn, sialoadhesin; NCAM, neural cell glycoprotein molecule; CHO, Chinese hamster ovary; PSGL-1, P-selectin glycoprotein ligand 1.
(24). Cells that were expressing CD43, as measured by flow cytometry using mAb S7 (BD Pharmingen, San Diego, CA), were used for experiments. The Chinese hamster ovary (CHO) cells stably transfected with human CD43 (CHO-CD43) and CHO-CD43 cotransfected with the core 2 β1,3-fucosyltransferase (CHO-CD43-C2F)T have been described before (26, 27) and were grown in DMEM/10% FCS containing G-418 (100 µg/ml) and/or hygromycin B (200 µg/ml). Expression of CD43 and the core 2 glycoform of CD43 was checked by flow cytometry as described previously using mAb IG10 (BD Pharmingen) and T305 (27). Cells (2 × 10^7/ml) were metabolically labeled overnight in glucose-low (10%) DMEM medium containing 10% dialyzed FCS and 1% normal FCS with 25–50 µCi/ml [3H]glucosamine as previously described (21).

**Immunoprecipitations**

Washed cells were lysed on ice in 1% Nonidet P-40 containing buffer (25 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, 0.5 mg/ml leupeptin, and 0.2 mg/ml aprotinin). After pelleting (10,000 × g for 10 min) insoluble material, the lysates were precleared for 2 h at 4°C with 50 µl of a 10% slurry of protein-A-Sepharose beads. Fractions were then incubated overnight with 5 µg of Fc protein at 4°C. Fifty microliters of protein-A-Sepharose was added and incubated for 1–2 h, and the beads were washed three times and eluted with SDS-sample buffer. In reprecipitation experiments, bound material was eluted for 3 min at 80°C in 15 µl of 3% SDS, followed by addition of 485 µl of lysis buffer and pre-clearing in protein-A-Sepharose, before proceeding with the next precipitation. Specificity of elution (used at 5 µg/incubation) included affinity-purified anti-peptide rabbit polyclonal Abs directed against the cytoplasmic tail of murine CD43 (28) and a rat IgG1 mAb, 2PH1, directed against murine P-selectin glycoprotein ligand 1 (PSGL-1) (29). Samples were resolved by 6.5% SDS-PAGE (nonreducing). Gels were fixed and radioactivity was visualized after soaking in Amersham Amplify (Amersham, Arlington Heights, IL) as recommended. Sialidase treatment was performed before lysis of the cells by incubation for 2 h at 37°C in 0.1 U/ml sialidase (Calbiochem, La Jolla, CA) in 25 mM HEPES-buffered RPMI 1640.

**Cell-binding assays**

Ninety-six-well plates (Immulon 3; Dynatec Laboratories, Chantilly, VA) were coated overnight at 4°C with 15 µg/ml anti-human IgG (Sigma, St. Louis, MO) in 0.1 M bicarbonate buffer (pH 9.6). After washing in PBS containing 0.25% BSA (PBS/BSA), blocking of nonspecific binding sites with 5% normal goat milk, and washing again, plates were incubated with the indicated Fc proteins for 1 h at room temperature. After washing, TK-1 cells (2 × 10^5 cells/ml) were added in 25 mM HEPES-buffered RPMI 1640 and incubated for 1 h at 37°C. COS cells (10^5 cells/ml) were labeled for 30 min at 37°C with 10 µg/ml 2',7'-bis-(2-carboxyethyl)-(5- and 6-) carboxyfluorescein-acetoxy methyl ester (Molecular Probes, Eugene, OR) dye in DMEM containing 20 mM HEPES. Cells were washed and resuspended in PBS/BSA, added (10^5 cells/well) to the coated plates, and incubated for 1 h at 37°C. The cells were fixed in 0.25% glutaraldehyde for 5 min, washed, and binding was quantified either by counting using an inverted microscope (TK-1) or by measuring fluorescence (excitation, 485 nm; emission, 530 nm) using a Cytofluor system (COS; Millipore, Bedford, MA). Data are expressed as mean ± SD.

**Results and Discussion**

Since our previous studies had shown that murine TK-1 T lymphoma cells demonstrate excellent sialoadhesin (Sn)-binding capacity (2), this cell line was chosen for the identification of potential counterreceptors for sialoadhesin. First, the capacity of TK-1 cells for binding to recombinant Sn-Fc protein was tested and compared with that of CD22 and CD33 (Fig. 1). A concentration-dependent binding to Sn-Fc was clearly observed. Binding to Sn was stronger than to CD22, whereas CD33 and control Fc protein (NCAM) did not show any TK-1 adhesion. To identify glycoprotein counterreceptors for Sn on TK-1 cells, Fc proteins were used in immunoprecipitation experiments employing lysates from TK-1 cells in which the glycans had been metabolically labeled with [3H]glucosamine. As can be seen in Fig. 2A, Sn-Fc selectively precipitated major glycoproteins of 240, 130, and 85 kDa, which was not seen with CD22-Fc or CD33-Fc. The recognition of TK-1 glycoproteins by Sn was sialic acid dependent: first, because it could in large part be prevented by pretreatment of cells with sialidase, and, second, because a mutation (R97A) in the first Sn Ig-like domain, which is known to abolish sialic acid-dependent binding (11), completely prevented recognition of TK-1 glycoproteins (Fig. 2B). The major sialylated glycoprotein on T cells is the 115- to 130-kDa sialomucin CD43 (leukosialin, sialophorin).

To test whether the dominant 130-kDa band precipitated by Sn-Fc represented CD43, an experiment was performed in which glycoproteins were precipitated with Sn-Fc, eluted, and reprecipitated with Sn-Fc or affinity-purified Abs against CD43. As can be seen in Fig. 3A, the 130-kDa glycoprotein could be selectively reprecipitated by anti-CD43 Ab and thus represents CD43. Similar reprecipitation experiments using the mAb 2PH1 identified the 240-kDa glycoprotein immunoprecipitated with the Sn-Fc protein as PSGL-1 (CD162; Fig. 3B). It was important to investigate whether surface-expressed CD43 and PSGL-1 can indeed mediate binding to Sn. Murine CD43 was expressed in COS cells and the binding to immobilized Sn-Fc was evaluated (Fig. 3C). Clearly, expression of CD43 led to an enhanced adhesion to Sn when compared with either sham- or complement receptor 1-transfected COS cells. All measurable binding was sialic acid dependent, since it could be reduced to background levels by sialidase pretreatment of the COS cells.
cells (data not shown). It is worth mentioning that the relatively high levels of expression in COS cells most probably are a good reflection of the situation in T cells, which carry the large number of 1–1.5 × 10^5 CD43 molecules/cell, covering an estimated 28% of the T cell surface area (30). In contrast to CD43, COS cells expressing PSGL-1 did not show enhanced adhesion to Sn-Fc compared with sham-transfected cells (data not shown).

In an earlier report, we have shown that both resting and activated T cells bind Sn, with the latter having a somewhat higher avidity (2). It has also been shown that the glycosylation of CD43 is modified during T cell activation, with resting T cells having simple unbranched core 1 structures, whereas activated T cells have branched core 2 glycans (31). This is caused by an activation-induced expression of the β1,6-GlcNAc transferase (also called the core 2 GlcNAc transferase, C2GnT), the key enzyme responsible for branching of O-linked glycan chains. To investigate whether both CD43 glycoforms were capable of binding Sn immunoprecipitation experiments were performed with CHO cells transfected with the human CD43 cDNA (CHO-CD43) and CHO-CD43 cells cotransfected with the β1,6-GlcNAc transferase (CHO-CD43-C2GnT). As can be seen in Fig. 4A, both CD43 transfectants expressed similar levels of CD43. The core 2 glycoform of the human CD43, identified by the T305 mAb, was expressed by a subpopulation (50–85%) of the CHO-CD43-C2GnT cells, probably reflecting a heterogeneous expression of the β1,6-GlnNAc transferase. As can be seen in Fig. 4B, the transfected CD43 (125 kDa) is selectively precipitated by Sn-Fc. From the double transfectants (CD43 plus C2GnT), two bands at 125 and 135 kDa were precipitated, representing the core 1 and core 2 forms of CD43, respectively. Thus, both glycoforms of CD43 are recognized by Sn.

Taken together, these results identify CD43 as a T cell counter-receptor for Sn. CD43 is a major glycoprotein, expressed primarily on T lymphocytes and myeloid cells, that carries the large number of 70–85 O-linked glycans, typically made up of the core 1-containing oligosaccharide NeuNAcα2,3Galβ1,3 (NeuNacc2,6)-GalNAc oligosaccharide (31–33). These CD43-associated glycans are likely to constitute a clustered ligand that binds, in a sialic acid-dependent fashion, to the V-set Ig-like domain of Sn. In the present experiments, branching of the oligosaccharide chains, to generate the core 2 structures predominantly found on activated T cells and myeloid cells, did not profoundly affect recognition. Considering the dimensions of Sn and CD43 (1, 34), which are each...
predicted to form elongated rod-like structures of 40 and 45 nm, respectively, it seems possible that these molecules mediate intercellular interactions at long distance and promote the initial physical contacts between macrophages and T cells. Whether this is important in the establishment of more firm contacts or, on the contrary, prevents the formation of short-range interactions remains to be investigated.

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