A Novel Mechanism of Carbohydrate Recognition by the C-type Lectins DC-SIGN and DC-SIGNR

SUBUNIT ORGANIZATION AND BINDING TO MULTIVALENT LIGANDS

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DC-SIGN and DC-SIGNR are cell-surface receptors that mediate cell-cell interactions within the immune system by binding to intercellular adhesion molecule-3. The receptor polypeptides share 77% amino acid sequence identity and are type II transmembrane proteins. The extracellular domain of each comprises seven 23-residue tandem repeats and a C-terminal C-type carbohydrate-recognition domain (CRD). Cross-linking, equilibrium ultracentrifugation, and circular dichroism studies of soluble recombinant fragments of DC-SIGN and DC-SIGNR have been used to show that the extracellular domain of each receptor is a tetramer stabilized by an α-helical stalk. Both DC-SIGN and DC-SIGNR bind ligands bearing mannose and related sugars through the CRDs. The CRDs of DC-SIGN and DC-SIGNR bind Man9GlcNAc2 oligosaccharide 130- and 17-fold more tightly than mannose, and affinity for a glycopeptide bearing two such oligosaccharides is increased by a further factor of 5- to 25-fold. These results indicate that the CRDs contain extended or secondary oligosaccharide binding sites that accommodate mammalian-type glycan structures. When the CRDs are clustered in the tetrameric extracellular domain, their arrangement provides a means of amplifying specificity for multiple glycans on host molecules targeted by DC-SIGN and DC-SIGNR. Binding to clustered oligosaccharides may also explain the interaction of these receptors with the gp120 envelope protein of human immunodeficiency virus-1, which contributes to virus infection.

DC-SIGN1 (dendritic cell-specific ICAM-3 grabbing nonintegrin; CD209), a novel cell-surface C-type lectin expressed on dendritic cells, has been shown to mediate interactions between dendritic cells and T-cells by binding ICAM-3 (1). These interactions are independent of lymphocyte function-associated antigen-1, which is the conventional ICAM-3 ligand. DC-SIGN also binds the gp120 envelope glycoprotein of human immunodeficiency virus-1 and facilitates viral infection in trans of target CD4+ T-cells (2, 3). The DC-SIGN gene is located on human chromosome 19p13, proximal to a gene encoding a closely related protein, termed DC-SIGNR (DC-SIGN-related) (4). Possible biological roles of DC-SIGNR have recently emerged, with reports demonstrating its capacity to bind ICAM-3, and also to gp120, mediating human immunodeficiency virus-1 infection in trans (5, 6). However, it is expressed on liver sinusoidal endothelium, the endothelium of lymph node sinuses, and placental capillary endothelium, rather than on dendritic cells. Low levels of DC-SIGN are co-expressed with DC-SIGNR on lymph node sinus endothelium.2

DC-SIGN and DC-SIGNR are type II transmembrane proteins that share 77% amino acid sequence identity (4). The extracellular domain of each consists of a series of seven and a half tandem repeats of a highly conserved sequence of 23 amino acids followed by a C-terminal C-type carbohydrate recognition domain (CRD). Both ICAM-3 and gp120 carry an abundance of N-linked high mannose oligosaccharides. Binding of ICAM-3 to DC-SIGN requires Ca2+, and interaction between DC-SIGN and gp120 is inhibited by mannann, mannoside, and EGTA (1, 2). These findings indicate that ligand binding is probably mediated through binding of carbohydrates by the CRD in a Ca2+-dependent manner. This hypothesis is consistent with the presence of residues believed to be necessary for mannose-binding to a C-type CRD in both DC-SIGN and DC-SIGNR (7).

It has been suggested that the repeating sequences between the transmembrane region and the CRDs mediate oligomer formation by forming an α-helical coiled-coil (2, 4). Similar structures mediate the oligomerization of other cell surface C-type lectins such as the mammalian hepatic asialoglycoprotein receptor, and the subunit organization of these oligomeric complexes is critical to their biological functions. Polymorphic variants of the DC-SIGN cDNA have been identified in which the length of the encoded neck regions differ, indicating that the cell-surface role of DC-SIGNR could be influenced by its specific neck structure (5).

In this study, soluble recombinant fragments of DC-SIGN and DC-SIGNR have been used to demonstrate that the extracellular domain of each molecule is a tetramer stabilized by an α-helical neck and that the individual CRDs possess high affinity for mannose-containing oligosaccharides. This information suggests that DC-SIGN and DC-SIGNR employ a novel mechanism of carbohydrate recognition to achieve specificity for their natural ligands by binding multiple high mannose oligosaccharides spaced at appropriate distances on the surface of a limited number of glycoproteins.

EXPERIMENTAL PROCEDURES

Materials—DNA restriction and modification enzymes were obtained from New England Biolabs. Oligonucleotides were supplied by Life

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2 E. J. Soilleux, personal communication.
A - DOMAIN ORGANIZATION

Transmembrane Region

Cytoplasmic tail

Neck

CRD

B - DC-SIGN

ATGGC MetAlaLySVal

62

Styl

Styl

C - DC-SIGNR

ATGGCCGGAACCC... MetAlaGiUArg...

250

PhΦM1

PΦM1

Fig. 1. Soluble fragments of DC-SIGN and DC-SIGNR produced by bacterial expression. Positions and 5’ sequences of oligonucleotides inserted to make suitable N-terminal segments are shown. In each case, the sequence Met-Ala is appended to the N terminus, and the methionine residue is removed during biosynthesis in E. coli.

Expression of Soluble DC-SIGN and DC-SIGNR Fragments—Plasmids containing the full-length cDNA of each protein were kindly provided by Elizabeth Soilleux (University of Cambridge). Constructs were generated from restriction fragments of the cDNAs ligated to synthetic double-stranded oligonucleotides designed to bridge the start of the desired coding point to proximal restriction sites within the cDNA sequence (Fig. 1). Standard recombinant DNA techniques were used throughout (13), and the integrity of the cloned fragments was checked by DNA sequencing. Modified cDNAs were inserted into the pET32a+ vector (12). Autoradiography was carried out on a PhosphoImager SI from Molecular Dynamics.

Inclusion bodies were isolated by centrifugation at 10,000 × g for 15 min at 4 °C, and the pellet was solubilized in 100 ml of 6 M guanidine-HCl containing 10 mM Tris-HCl, pH 7.0, and 0.1% β-mercaptoethanol, by brief sonication followed by gentle rotation for 20 min at 4 °C. The mixture was centrifuged at 157,000 × g for 30 min at 4 °C in a Beckman 55.2Ti rotor and the supernatant was diluted with loading buffer (1.25 M NaCl containing 25 mM Tris-HCl, pH 7.8, and 25 mM CaCl2) by slow addition with stirring. The diluted mixture was dialyzed against 6 volumes of loading buffer with 2 buffer changes. After dialysis, insoluble precipitate was removed by centrifugation at 142,000 × g for 1 h at 4 °C in a Beckman 45Ti rotor and the supernatant was loaded onto a Man-Sepharose column (1–5 ml) pre-equilibrated with loading buffer. The column was washed with loading buffer (5–10 column volumes) and eluted with 4–5 column volumes of eluting buffer (1.25 M NaCl containing 25 mM Tris-HCl, pH 7.8, and 2.5 mM EDTA). Fractions were analyzed by SDS-PAGE. The identity of each protein was confirmed by N-terminal sequencing.

Ion-exchange Chromatography—Further purification of DC-SIGN and DC-SIGNR fragments was carried out by ion-exchange chromatography on a Mono-Q column (Amersham Pharmacia Biotech) equilibrated in 50 mM Tris-HCl, pH 7.8, at a flow rate of 1 ml/min at room temperature. Proteins were eluted from the column using a 500 mM NaCl gradient in the same buffer over 30 min, and 1 ml fractions were collected. Absorbance was monitored at 280 nm, and fractions were analyzed by SDS-PAGE.

Cross-linking Analysis—Proteins for cross-linking were first dialyzed against 100 mM HEPES pH 7.5. Cross-linking was performed for 1 h at room temperature in the presence and absence of 10 mM CaCl2 using the homobifunctional reagent bis(sulfosuccinimidyl) suberate (15). Reactions were stopped by addition of double-strength SDS gel loading buffer containing 1% β-mercaptoethanol, followed by heating at 100 °C for 5 min. Samples were analyzed by SDS-PAGE.

Analytical Ultrasound Centrifugation—Sedimentation equilibrium experiments were carried out in a Beckman XL-A analytical ultracentrifuge equipped with absorbance optics using an An60Ti rotor following protocols described previously (16). Analysis of the extracellular fragments of DC-SIGN and DC-SIGNR was carried out at rotor speeds of 8,000 and 10,000 rpm, with 24 h delays prior to the start of the scan sessions. For the CRD fragments, rotor speeds of 15,000 and 20,000 rpm were used. Equilibrium data were collected at either 232, 280, or 290 nm in step scan mode using a separation of 0.001 cm. Theoretical partial specific volumes of the proteins were determined from their amino acid compositions (17). For each protein, the weight-averaged molecular mass at concentrations throughout the sample cells was determined using software provided by the manufacturer.

Circular Dichroism Spectroscopy—Circular dichroism spectra were measured on a Jasco J6000 spectropolarimeter using 200-μl samples in a 1 mm quartz cuvette at room temperature. Ten scans were carried out on each sample from 190 to 250 nm with a bandwidth of 2 nm and a scan rate of 20 nm/min. Protein concentrations were determined by alkaline ninhydrin assay (18).

Sugar-Competition Assays—Proteins were immobilized on Immulon-4 polystyrene wells in 96-well plates, by overnight incubation of 250 μg/ml stock solution at 4 °C (50 μl per well). Following washing with loading buffer, the plates were blocked with loading buffer containing 5% BSA (500 μl per well) for 2 h at 4 °C and washed again with loading buffer. The plates were incubated for 2 h at 4 °C with loading buffer containing 5% BSA, 10 mM 125I-ManBSA reporter ligand, and competing sugar (1 μg per well). After three washes with loading buffer, wells were counted in a Wallace 1420 Wizard γ counter. Duplicate samples were run in each assay, and data were fitted using a nonlinear least squares fitting algorithm (SigmaPlot) to determine concentrations of competing ligand required for 50% inhibition of reporter ligand binding (Ks).

Direct Binding Assays—Polystyrene plates were coated with protein and blocked with BSA as for the competition assays. 125I-ManBSA was diluted with nonradioactive ManBSA to a final specific activity of 1–2 × 105 cpm/μg and 2-fold serial dilutions, prepared in loading buffer containing 5% BSA, were incubated in the plates (100 μl per well) for 2 h at 4 °C. Plates were washed three times with loading buffer and the radioactivity remaining in the wells was counted. Assays were run in duplicate and dissociation constants (Kd) were calculated by fitting the data to an equation for simple, saturable bindingsuperimposed on a linearly increasing background of nonspecific binding as described previously (19). Glycopeptide Preparation and Cross-linking—Glycopeptide carrying a Man3GlcNAc2 oligosaccharide was prepared by digesting soybean agglutinin (~80 mg in 30 ml of 1% NH4CO3) for 16 h at 37 °C with 4
mg of subtilisin. Digested material was treated with phenylmethylsulfonyl fluoride (0.03% w/v) and lyophilized before being redissolved in 0.5 ml of 1% acetic acid and loaded onto a Sephadex G-25 column (7.5 ml). Fractions (0.5 ml) were collected in 1% acetic acid, and aliquots of 10 μl were assayed using the anthrone method calibrated with methyl α-mannoside standards (from 10 to 40 μg) (20). Glycopeptide was lyophilized and redissolved in 0.5 ml of 50 mM sodium phosphate, pH 7.5, containing 1 mM EDTA, followed by treatment with a 5-fold molar excess of N-succinimidyl S-acetylthioacetate (21), added as a 10× concentrated reagent stock in dimethyl sulfoxide, for 30 min at room temperature. Derivatized product was separated from the unreacted reagent by chromatography on Sephadex G-25 as above before being lyophilized, redissolved in 0.5 ml of deacetylation buffer (50 mM sodium phosphate, pH 7.5, containing 1 mM EDTA), followed by treatment with a 5-fold molar excess of Bolton and Hunter reagent (100 μCi) for 30 min at room temperature. Following incubation with the indicated concentrations of bis(sulfosuccinimidyl) suberate (μM), the samples were resolved on a 10% acrylamide gel that was stained with Coomassie Blue.

Neoglycolipid Overlays—Neoglycolipids were prepared by conjugation of oligosaccharides from bovine RNase B to phosphatidylcholamine dipalmitate in the presence of sodium cyanoaurate (22). High performance thin-layer chromatography of the neoglycolipids was carried out using a chloroform/methanol/water (105:100:28 v/v) solvent mixture. Chromatograms were dried and fixed by a 30-s incubation in a chloroform/methanol/water (1:1:1 v/v) mixture. Chromatograms were stained with Coomassie Blue.

RESULTS

Fragments of both DC-SIGN and DC-SIGNR, representing the CRDs alone and the complete extracellular domains, were expressed in E. coli and purified on Man-Sepharose (Fig. 2). Although the CRD fragments of both DC-SIGN and DC-SIGNR are retarded on the columns, they elute predominantly in the wash fractions and thus bind mannose relatively weakly. The extracellular domain fragments of DC-SIGN and DC-SIGNR bind to the columns with greater affinity and are retained in the presence of Ca²⁺ and elute with EDTA.

Physical Characterization—The oligomeric states of the DC-SIGN and DC-SIGNR fragments were examined by chemical cross-linking in the presence and absence of Ca²⁺ (Fig. 3). Dimer, trimers, and tetramers of the extracellular domain fragments were detected at increasing concentrations of cross-linking reagent. The pattern observed was the same in both the presence and absence of Ca²⁺ and was almost identical for DC-SIGN and DC-SIGNR. These results suggest that the extracellular domain of each protein exists in solution as a tetramer. Similar treatment of the CRDs produced only a single band across the entire range of cross-linking reagent concentrations, indicating that these fragments are monomers.

The cross-linking results were confirmed by analytical ultracentrifugation (Fig. 4). At loading concentrations ranging from 1 and 5 × 10⁷ cpm/ml in loading buffer containing 2% BSA) for a further 2 h at room temperature. Following four wash steps with loading buffer, the chromatograms were dried, and the radiolabeled proteins visualized using a phosphorimager.
0.05 to 1.5 mg/ml, the extracellular domain fragment of DC-SIGN is a single stable species with an apparent molecular mass corresponding closely to the theoretical value for a tetramer. A similar result was obtained for DC-SIGNR, analyzed over a loading concentration range of 0.02–0.8 mg/ml, again indicating the existence of a tetramer. In each case, parallel experiments using protein dialyzed into Ca²⁺-free buffer yielded similar results, demonstrating that tetramer formation is Ca²⁺-independent. Sedimentation data for the CRD fragments of both DC-SIGN and DC-SIGNR confirm that each CRD exists as a monomer. Thus, the neck regions of DC-SIGN and DC-SIGNR are essential for oligomer formation.

Secondary structures of the neck regions of DC-SIGN and DC-SIGNR were determined by circular dichroism spectroscopy using the difference between spectra of the extracellular domain and CRD fragments to define the spectrum of the neck (Fig. 5). The results for both DC-SIGN and DC-SIGNR are consistent with an α-helical conformation, as indicated by the characteristic maximum at 193 nm and minima at 208 nm and 223 nm.

The close similarity between the sequences of the neck regions in DC-SIGN and DC-SIGNR suggests that they could form hetero-oligomers (4). The extracellular domain fragments of DC-SIGN and DC-SIGNR elute from a Mono-Q column at slightly different salt concentrations, and the two polypeptides can be resolved on a 15% polyacrylamide gel. When these fragments of DC-SIGN and DC-SIGNR are allowed to renature together, some of the protein elutes from the Mono-Q column at an intermediate elution position, suggesting that hetero-oligomers are formed. When examined by analytical gel filtration, these hetero-oligomers behave identically to the homo-oligomers, indicating that mixed tetramers are formed rather
than higher order oligomers (data not shown).

Monosaccharide Binding Activity—The sugar-binding properties of the extracellular domains of DC-SIGN and DC-SIGNR were investigated using solid phase assays in which monosaccharides compete with \( \text{\textsuperscript{125}I-Man}_{30}\text{-BSA} \) for binding to the immobilized protein (Table I). Both DC-SIGN and DC-SIGNR bind mannose better than they bind galactose, as expected from earlier analysis of the CRD sequences. However, the margin of specificity is narrower than for other C-type lectins, such as MBP (24). Binding of galactose to mannose-specific C-type CRDs can occur through interactions with the C-1 and C-2 hydroxyl groups of the free sugar (25). To eliminate such non-physiological binding, further competition experiments were performed with methyl glycosides. Inhibition by methyl \( \alpha\)-galactoside is 27- to 34-fold less effective than inhibition by methyl \( \alpha\)-mannoside, indicating a preference for methyl \( \alpha\)-mannoside comparable with serum MBP. These results suggest that DC-SIGN and DC-SIGNR, like other C-type lectins, distinguish monosaccharides through recognition of the C-3 and C-4 hydroxyl groups, and the preferred ligands have an equatorial arrangement at these positions.

Both DC-SIGN and DC-SIGNR show preference for axial C-2 substituents, as they interact with glucose and GlcNAc more weakly than they interact with mannose. For both proteins, affinity is increased by replacement of the axial hydroxyl group of mannose with the \( \text{\textsuperscript{N}}\text{Acetylmannosamine} \). However, binding of GlcNAc to DC-SIGNR is somewhat tighter than binding to DC-SIGN. Also, DC-SIGN has higher affinity for L-fucose than for mannose whereas DC-SIGNR has higher affinity for mannose than for L-fucose.

High Affinity Binding to High Mannose Oligosaccharides—The \( \text{\textsuperscript{Man}_9\text{GlcNAc}_2} \) oligosaccharide of soya bean agglutinin was used as a convenient, homogeneous ligand to investigate the interaction of DC-SIGN and DC-SIGNR with oligosaccharides similar to those found on their natural ligands. On an equimolar basis, this oligosaccharide competes for binding to the extracellular domain of DC-SIGN 24-fold more effectively than does mannose (Fig. 6A). This type of cluster effect, in which affinity for a multivalent sugar ligand is enhanced compared with simple monosaccharides, has been observed for other C-type lectins (26). The increased affinity is often related to formation of lectin oligomers in which multiple CRDs bind to multiple terminal sugar residues in the ligands. However, the results in Fig. 6B demonstrate that a comparable cluster effect for DC-SIGNR is observed for the monomeric CRD. A slightly more pronounced cluster effect is observed for the extracellular domain of DC-SIGN and a still greater effect is observed for the CRD (Table II). The fact that oligosaccharides bind with high affinity to an individual CRD in DC-SIGN and DC-SIGNR indicates that the CRD must make additional contacts with the oligosaccharide beyond the primary mannose-binding site. The fact that the cluster effect for the extracellular domain fragment of DC-SIGN is actually less than for the isolated CRD suggests that some of these contacts must be occluded when the CRDs are assembled in the tetramer.

Enhanced affinity for the \( \text{\textsuperscript{Man}_9\text{GlcNAc}_2} \) oligosaccharide compared with mannose was also demonstrated using a fluid-phase assay protocol (27), confirming the solid-phase data and ruling out the possibility that the cluster effect within the CRDs could result from the immobilization protocol (data not shown). For comparison, solid-phase competition assays were also performed using plates coated with a trimeric fragment of rat MBP. In contrast to the DC-SIGN and DC-SIGNR results, the relative affinities of MBP for \( \text{\textsuperscript{Man}_9\text{GlcNAc}_2} \) and mannose are almost indistinguishable, confirming previous studies (28). These findings highlight a major difference in the pattern of carbohydrate recognition displayed by these two classes of mannoside-binding molecules.

Because the oligosaccharides on natural ligands for DC-SIGN and DC-SIGNR include a range of high mannose struc-
TABLE II
Binding constants for oligosaccharide and neoglycoprotein binding to DC-SIGN and DC-SIGNR

| Oligosaccharide Type | DC-SIGN ECD | DC-SIGN CRD | DC-SIGNR ECD | DC-SIGNR CRD | MBP | Neoglycolipid |
|----------------------|-------------|-------------|-------------|-------------|-----|--------------|
| K<sub>i</sub>, Monomer | 7.7 ± 1.0 | 2.1 ± 0.3 | 2.4 ± 0.1 | 3.2 ± 0.3 | 1.9 ± 0.1 | 0.3 ± 0.01 |
| K<sub>i</sub>, Man<sub>9</sub>GlcNAc<sub>2</sub> | 0.21 ± 0.01 | 0.016 ± 0.001 | 0.11 ± 0.01 | 0.19 ± 0.01 | 1.2 ± 0.2 | 37 ± 6 |
| K<sub>i</sub>, Dimeric glycopeptide | 3.05 | 131 ± 26 | 24 ± 3 | 17 ± 2 | 1.6 ± 0.3 | 0.35 |
| K<sub>i</sub>, Man<sub>9</sub>GlcNAc<sub>2</sub> | 0.039 ± 0.006 | 0.0031 ± 0.0003 | 0.0043 | 0.0085 | -- | 5.4 ± 1.0 |
| K<sub>i</sub>, Dimeric glycopeptide | 0.016 ± 0.001 | 0.0031 ± 0.0003 | 5.2 ± 0.8 | 22 ± 1 | -- | 32 ± 7 |

* ECD, extracellular domain.
* Not determined.

A major issue to be addressed is how the properties of DC-SIGN and DC-SIGNR are tailored to mediate specific recognition of ICAM-3 among the many cell-surface glycoproteins that display similar oligosaccharides. Part of the selectivity for specific glycoprotein ligands resides within the CRDs. These domains are unique among those of the C-type lectin family in that they show a pronounced cluster effect in their monomeric form. Ca<sup>2+</sup> dependence and selectivity of monosaccharide binding to DC-SIGN and DC-SIGNR, along with sequence comparisons, suggest that the primary binding site is analogous to that seen in other C-type CRDs. Such sites generally accommodate terminal sugar residues by direct ligation to bound monosaccharides. In either case, the K<sub>i</sub> values determined here suggest that the CRDs bind high mannose-type oligosaccharides with submillimolar affinity.

Further selectivity for glycoproteins that bear multiple high mannose oligosaccharides such as ICAM-3 and gp120 can be achieved by grouping four CRDs in a tetramer. The results comparing the binding of dimerized soybean agglutinin glycopeptide and Man<sub>9</sub>GlcNAc<sub>2</sub>-BSA suggest that high mannose oligosaccharides would need to be placed some distance apart on a glycoprotein surface in order to interact with multiple CRDs in the tetramer. Thus, selective binding to ligands may reflect the presence of a specific geometric arrangement of high mannose oligosaccharides. Glycans on gp120 of human immunodeficiency virus presumably have a similar arrangement in order to increase viral infectivity through interactions with DC-SIGN and DC-SIGNR.

Compared with MBP, DC-SIGN and DC-SIGNR employ mannose recognition in a very different way to perform distinct immunological roles. MBP binds to large glycosylated surfaces in order to direct an innate immune response to foreign organisms (32). Each CRD in MBP binds a single terminal mannose residue and high avidity binding is only achieved by the clustering of CRDs in trimeric subunits. The large inter-CRD spacing of mannose-binding sites allows binding to widely spaced residues such as those found on the surfaces of bacterial and...
fungal cells, whereas endogenous mannose-containing glycans are comparatively poor ligands because of the relatively short spacing between terminal residues. In contrast, DC-SIGN and DC-SIGNR may recognize multiple self-oligosaccharides in specific arrangements on the surfaces of select glycoproteins. These recognition events contribute to the formation of host cell-cell interactions, and the flow of information within the immune system.

Formation of tetramers by DC-SIGN and DC-SIGNR contrasts with other multimeric C-type lectins, such as MBP and asialoglycoprotein receptors, that form trimers stabilized by regions of α-helical coiled-coil adjacent to the CRDs. This diversification of oligomeric state within the C-type lectin family has probably emerged in order to accommodate distinct patterns of carbohydrate recognition. The neck structures of DC-SIGN and DC-SIGNR are unusual, as only the first half of each 23 amino acid repeat displays a discernable pattern of hydrophobic residues, spaced at regular intervals, that is observed in most coiled-coils (33, 34). In addition, each repeat contains a proline residue. Nevertheless, the circular dichroism experiments indicate that the repeats fold in an α-helical conformation. Nonetheless, these two molecules may present a novel form of tetrameric coiled-coil motif.

Hetero-oligomeric complexes can be formed between the extracellular domain fragments of DC-SIGN and DC-SIGNR. However, these could only be formed in vitro in cell types where the two receptors are co-expressed, such as lymph node sinus endothelium2 and placental capillary endothelium (6). Moreover, possible expression of polymorphic variants of DC-SIGNR, in which neck lengths differ, would be likely to prevent such hetero-oligomer formation (5).

Based on the close sequence similarity between DC-SIGN and DC-SIGNR and the physical studies presented here, it can be concluded that these two proteins are very similar in overall structure. They also show similar ligand binding characteristics with the various test ligands employed. However, subtle differences in carbohydrate binding are evident, both at the monosaccharide level and in the way that the CRDs and oligomers interact with clustered ligands. For example, it is particularly striking that close spacing of two oligosaccharides causes substantially greater increase in affinity for DC-SIGNR than for DC-SIGN. Although the best characterized natural ligands, ICAM-3 and gp120, are common to both DC-SIGN and DC-SIGNR, the receptors differ in their ability to facilitate infection by human immunodeficiency virus-1 in vitro (6), which might reflect subtle differences in their relative interaction with gp120.

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