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Molecular Basis of the Differences in Binding Properties of the Highly Related C-type Lectins DC-SIGN and L-SIGN to Lewis X Trisaccharide and *Schistosoma mansoni* Egg Antigens*

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The dendritic cell-specific C-type lectin DC-SIGN functions as a pathogen receptor that recognizes *Schistosoma mansoni* egg antigens through its major glycan epitope Galβ1,4(Fucα1,3)GlcNAc (Le^x). Here we report that L-SIGN, a highly related homologue of DC-SIGN found on liver sinusoidal endothelial cells, binds to *S. mansoni* egg antigens but not to the Le^x epitope. L-SIGN does bind the Lewis antigens Le^a, Le^b, and Le^y, similar as DC-SIGN. A specific mutation in the carbohydrate recognition domain of DC-SIGN (V351G) abrogates binding to all Lewis antigens. In L-SIGN Ser^{363} is present at the corresponding position of Val^{351} in DC-SIGN. Replacement of this Ser into Val resulted in a "gain of function" L-SIGN mutant that binds to Le^x, and shows increased binding to the other Lewis antigens. These data indicate that Val^{351} is important for the fucose specificity of DC-SIGN. Molecular modeling and docking of the different Lewis antigens in the carbohydrate recognition domains of L-SIGN, DC-SIGN, and their mutant forms, demonstrate that Val^{351} in DC-SIGN creates a hydrophobic pocket that strongly interacts with the Fucα1,3/4-GlcNAc moiety of the Lewis antigens. The equivalent amino acid residue Ser^{363} in L-SIGN creates a hydrophilic pocket that prevents interaction with Fucα1,3-GlcNAc in Le^x but supports interactions with the Fucα1,4-GlcNAc moiety in Le^a and Le^b antigens. These data demonstrate for the first time that DC-SIGN and L-SIGN differ in their carbohydrate binding profiles and will contribute to our understanding of the functional roles of these C-type lectin receptors, both in recognition of pathogen and self-glycan antigens.

Dendritic cell-specific intercellular adhesion molecule-3 (ICAM-3)[1]-grabbing nonintegrin (DC-SIGN, CD209) is a type II membrane C-type lectin with a short amino-terminal cytoplasmic tail and a single carboxyl-terminal carbohydrate recognition domain (CRD). DC-SIGN is a cell adhesion receptor that mediates interactions between dendritic cells (DCs) and resting T cells through binding to ICAM-3 and supports trans-endothelial migration through interaction with ICAM-2 (1, 2). DC-SIGN has also been described as a pathogen receptor. It binds human immunodeficiency virus type 1 (HIV-1) gp120 and facilitates the transport of HIV from mucosal sites to draining lymph nodes where infection of T-lymphocytes occurs (3). Recent reports show that DC-SIGN binds to other viruses like hepatitis C (4, 5), Ebola (6), cytomegalovirus (7), and Dengue (8), as well as other pathogens such as *Mycobacterium* (9–11), *Leishmania* (12, 13), and *Candida albicans* (14). Recently we showed that DC-SIGN binds soluble egg antigens (SEA) of the helminth parasite *Schistosoma mansoni* through the Lewis x (Le^x, CD15) glycan antigen (15). This binding to Le^x and SEA was abrogated by mutation of the Val at position 351 in the CRD of DC-SIGN.

Liver/lymph node-specific ICAM-3-grabbing nonintegrin (L-SIGN/CD209L/DC-SIGN-R) is a human homologue of DC-SIGN. L-SIGN shares 77% amino acid sequence identity with DC-SIGN and has functional similarity by recognizing ICAM-2, ICAM-3, and HIV-1 gp120. In addition, elucidation of the crystal structures of the CRDs of both DC-SIGN and L-SIGN, in combination with binding studies, revealed that both receptors recognize high mannose oligosaccharides (16, 17). Whereas for DC-SIGN increasing evidence indicates that its major ligands are α3/α4-fucosylated glycans (15, 18, 19), no data so far indicate a role for L-SIGN in the binding of fucosylated oligosaccharides. L-SIGN is not expressed by DCs but is expressed by endothelial cells present in lymph node sinuses, capillary endothelial cells of the placenta, and liver sinusoidal endothelial cells (20–23). Because liver sinusoidal endothelial cells function as a liver-resident antigen presenting cell population (24), it is of particular interest to investigate whether L-SIGN plays a role in the recognition and uptake of glycosylated schistosome egg antigens that are secreted by eggs trapped in the liver of schistosome-infected hosts.

Here we demonstrate that L-SIGN interacts with *S. mansoni* SEA but recognizes another glycoprotein fraction than DC-SIGN does, suggesting that L-SIGN does not recognize Le^x. Indeed, binding assays with L-SIGN demonstrated a lack of binding of L-SIGN to neoglycoconjugates carrying Le^x but showed that L-SIGN recognizes other fucosylated glycans, *i.e.*

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[1] The abbreviations used are: ICAM-3, intercellular adhesion molecule-3; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin; Le^x, Lewis x, Galβ1,4(Fucα1,3)GlcNAc; Le^a, Lewis a, Galβ1,3(Fucα1,4)GlcNAc; Le^y, Lewis y, Fucα1,2Galβ 1,4(Fucα1,3)GlcNAc; Le^b, Lewis b, Fucα1,2Galβ 1,3(Fucα1,4)GlcNAc; DC, dendritic cell; CRD, carbohydrate recognition domain; HIV, human immunodeficiency virus; gp, glycoprotein; SEA, soluble egg antigens; L-SIGN, liver/lymph node-specific ICAM-3-grabbing nonintegrin; PAA, polyacrylamide; SEA-bio, biotinylated SEA; sLe^x, sialyl Le^x.

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Lewis a (Le^a), Lewis b (Le^b), and Lewis y (Le^y). Site-specific mutagenesis of the amino acid residue Ser^{363} in L-SIGN into a Val, as is present in DC-SIGN at this position, resulted in a "gain of function" mutant that binds to neoglycoconjugates carrying Le^x and shows increased binding to the other Lewis antigens. Molecular modeling of the CRDs of the wild-type and mutant lectins demonstrated that Val^{351} in DC-SIGN, which is lacking in L-SIGN, may be critically involved in the binding to Le^x and Le^a by creating a strong hydrophobic contact with the fucose. These data show for the first time that DC-SIGN and L-SIGN differ in their carbohydrate recognition profiles and propose a molecular model that explains the observed differential binding of these lectins to fucosylated glycan antigens.

EXPERIMENTAL PROCEDURES

Antibodies and Neoglycoconjugates—The following antibodies were used, AZN-D1 (IgG1, anti-DC-SIGN), AZN-D2 (anti-DC-SIGN/anti-L-SIGN) (1, 22), and anti-LDN monoclonal antibody SMLDN1.1 (25). Crude *S. mansoni* SEA extract was centrifuged for 90 min at 100,000 × *g* at 4 °C and sterilized by passing through a 0.2 μM filter, essentially as described by Nyame *et al.* (26). Neoglycoconjugates, containing glycans multivalently coupled to biotinylated polyacrylamide (PAA) were from Lectinity (Finland).

Mutagenesis—Mutations in the cDNA encoding L-SIGN were generated using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) and pRc/CMV-L-SIGN plasmid according to the manufacturer's protocol. Plasmid sequencing confirmed the introduction of a mutation in the Ser residue at position 363 into Val (S363V) or Gly (S363G). Stable K562 cell lines expressing L-SIGN mutants were generated by the transfection of K562 cells with 10 μg of plasmid as described previously (3, 27, 28). Positive cells were sorted several times with the antibody AZN-D2 to obtain stable transfectants with similar expression levels (see Fig. 3*B*, >85%).

Binding Assay—Stable K562 cells expressing different wild-type and mutant C-type lectins (5×10^4 cells) were incubated in a total volume of 25 μl with biotinylated PAA-linked glycoconjugates (5 μg/ml) in adhesion buffer (20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.5% bovine serum albumin) for 30 min at 37 °C. Cells were washed with adhesion buffer and incubated with streptavidin-Alexa Fluor 488 secondary antibody (Molecular Probes, Inc., Eugene, OR) for 20 min at room temperature and analyzed using flow cytometry in the FL-1 channel (FACScan, BD Biosciences).

Fluorescent Bead Adhesion Assay—For measuring SEA binding to whole cells, a bead adhesion assay was used as described previously (27). Streptavidin was covalently coupled to TransFluorSpheres, fluorescent beads with a size of 1.0 μm and fluorescence at 488/654 nm (Molecular Probes Inc.). The streptavidin-coated beads were incubated with a biotinylated $F(ab')_2$ fragment of goat anti-mouse IgG (6 μg/ml, Jackson ImmunoResearch) followed by an overnight incubation at 4 °C with anti-LDN monoclonal antibody. The beads were washed and incubated with 1 μg/ml SEA overnight at 4 °C (15). Alternatively, SEA was biotinylated with EZ-link™ NHS-LC-LC-biotin, according to the manufacturer's protocol (Pierce). Biotinylated SEA (SEA-bio) was directly coupled to the streptavidin-coated fluorescent beads. HIV gp120 fluorescent beads were prepared as described previously (3). Briefly, for the fluorescent beads adhesion assay 50×10^3 cells were resuspended in adhesion buffer (20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.5% bovine serum albumin). Ligand-coated fluorescent beads (20 beads/cell) were added to the cells, and the suspension was incubated for 45 min at 37 °C. Cells were washed, and adhesion was determined using flow cytometry (FACScan) by measuring the percentage of cells that had bound fluorescent beads in the FL-3 channel.

Molecular Modeling—The starting coordinates of human DC-SIGN and L-SIGN (17) were taken from the Protein Data Bank (29) using files with code 1K9I and 1K9J, respectively. Using the Sybyl software (Tripos Inc., St. Louis, MO), the structures were edited to contain only one protein monomer together with calcium ions. Protein hydrogen atoms were added, the peptide atoms partial charges were calculated using the Pullman procedure, and the calcium ions were given a charge of 2. The positions of the hydrogen atoms were refined with the use of the Tripos force field (30). Lewis oligosaccharides were built from a data base of three-dimensional structures of monosaccharides with their glycosidic torsion angles corresponding to the lowest energy conformation determined previously (31). Atom types and charges for oligosaccharides were defined using parameters developed for carbohydrates (32).

Docking of oligosaccharides in the binding sites was performed by testing the several possible orientations of the fucose hydroxyl groups in the coordination sphere of the calcium ion. Energy minimization was performed using the Tripos force field (30) with geometry optimization of the sugar and the side chains of amino acids in the binding sites. A distance-dependent dielectric constant was used in the calculations. Energy minimizations were carried out using the Powell procedure until a gradient deviation of 0.05 $kcal \cdot mol^{-1} \cdot Å^{-1}$ was attained.

RESULTS

L-SIGN Shows Binding to S. mansoni SEA—In a previous study (15), we showed that DC-SIGN binds *S. mansoni* SEA through the recognition of Le^x antigens. To investigate the binding properties of L-SIGN to SEA, we used a fluorescent bead adhesion assay with K562 transfectants stably expressing L-SIGN or DC-SIGN (Fig. 1*A*). Streptavidin-coated fluorescent beads were precoated with monoclonal antibodies recognizing LDN glycan antigens (25) that occur on a SEA glycoprotein fraction that simultaneously carries Le^x glycan antigens (15). These LDN-coated fluorescent beads were then used to capture SEA and incubated with K562 cells expressing L-SIGN as described previously for DC-SIGN (15). The LDN-captured SEA beads interacted with K562 cells expressing DC-SIGN; however we could not observe any binding to L-SIGN (Fig. 1*C*). However, by using this approach only a fraction of the SEA was coated on the beads (*i.e.* only SEA containing LDN glycans also carrying Le^x (15)). To investigate binding of L-SIGN to the whole SEA population, SEA was biotinylated and directly coated on streptavidin-coated fluorescent beads. Interestingly, K562 cells expressing L-SIGN bound to beads carrying biotinylated SEA (Fig. 1, *B* and *C*). In agreement with previous results L-SIGN was able to bind HIV-1 gp120 beads in a manner similar to DC-SIGN (Fig. 1*C*) (22). The binding of beads coated with biotinylated SEA to both DC-SIGN and L-SIGN could be blocked by the anti-DC-SIGN/L-SIGN antibody AZN-D2 and by EDTA, which removes the Ca^{2+} ions that are essential for carbohydrate binding, indicating that the CRDs of the lectins are involved in the binding of SEA (Fig. 1*D*). These data show that L-SIGN binds *S. mansoni* SEA but recognizes a different subset of SEA compared with DC-SIGN, which suggests that L-SIGN differs from DC-SIGN in its ability to bind Le^x glycan antigens.

L-SIGN Does Not Bind to Le^x but Recognizes Other Lewis Antigens—Because our previous data indicated that the trisaccharide Le^x on schistosome SEA is a ligand for DC-SIGN, we next investigated whether L-SIGN binds to Le^x glycans linked to biotinylated PAA. The results in Fig. 2 show that K562 cells expressing L-SIGN did not bind Le^x-PAA in contrast to K562 cells expressing DC-SIGN that strongly bound to Le^x-PAA. The binding of DC-SIGN to Le^x-PAA could be inhibited by a blocking antibody against DC-SIGN (AZN-D2) and by the calcium chelator EGTA (Fig. 2*B*).

It has been described that the CRD of DC-SIGN binds two Ca^{2+} ions and that amino acids in close contact with these Ca^{2+} binding sites are essential for ligand binding (1, 28). The amino acid sequences of the CRD domains of L-SIGN and DC-SIGN show a high identity (Fig. 3*A*). Recently we showed that mutation of amino acid residue Val^{351} into a Gly in DC-SIGN (Fig. 3*A*, V351G) abrogated the binding to SEA and Le^x, whereas binding to HIV gp120 was not affected (15, 28). This suggests that Val^{351} within the CRD of DC-SIGN is important for the binding of DC-SIGN to Le^x. In L-SIGN, a Ser is located at the position of Val in DC-SIGN. To determine the molecular basis for the difference in carbohydrate specificity between L-SIGN and DC-SIGN, two mutations were made in the CRD domain of L-SIGN in which Ser at position 363 is converted into a Val (S363V) or alternatively into a Gly (S363G) (Fig. 3*A*). The carbohydrate binding capacity of K562 cells expressing these

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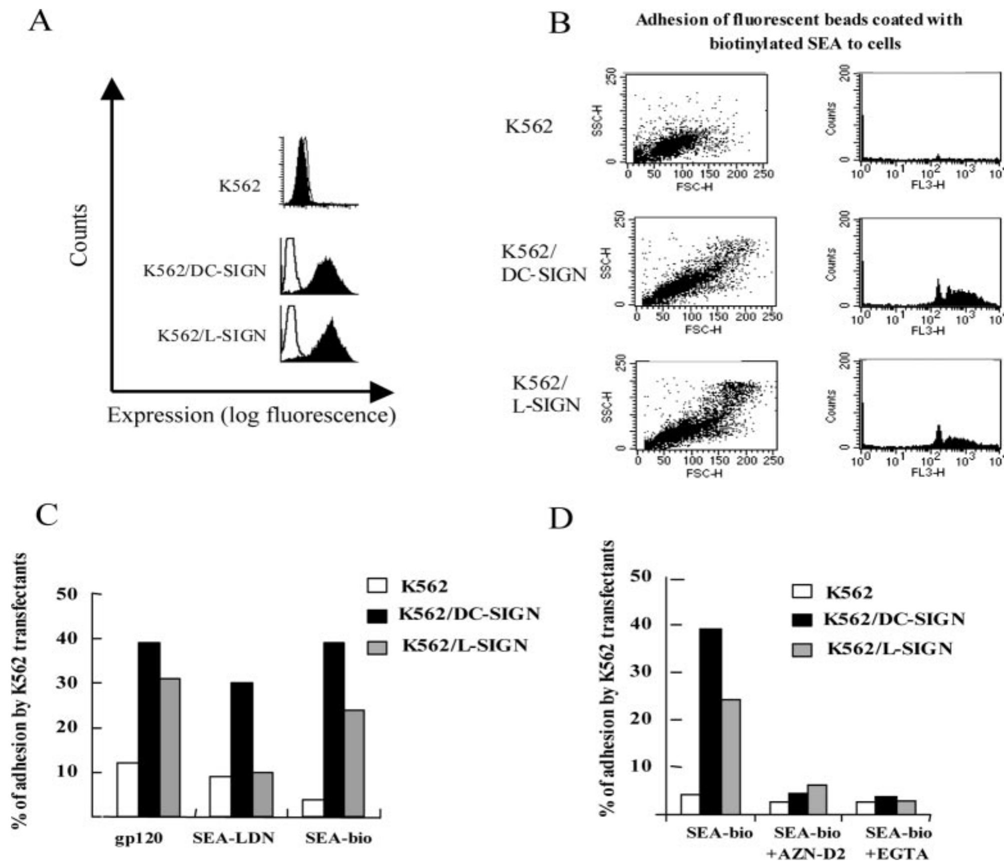

FIG. 1. **Fluorescent beads adhesion assays of *S. mansoni* SEA with K562 cells expressing L-SIGN or DC-SIGN.** *A*, K562 cells stably transfected with L-SIGN or DC-SIGN have similar expression levels as determined by FACScan analysis using monoclonal antibody AZN-D2 that recognizes a common epitope on L-SIGN and DC-SIGN. *B*, SEA was biotinylated and coupled to streptavidin-coated fluorescent beads (SEA-bio). Binding of the beads to the cells was measured by FACScan analysis using Cellquest (BD Biosciences). The dotplots (*left panel*) show the forward-side scatter (*FSC*) of the cells in the presence of SEA-bio. The adhesion of the beads to the cells is measured in the *FL-3* channel and shown as histoplots (*right panel*). *C*, binding of HIV-1 gp120- and SEA-coated beads to the cells was measured using the fluorescent bead adhesion assay as in B and shown as % binding. In addition to SEA-bio (see B), fluorescent beads coupled to an anti-LDN-monoclonal antibody (25) were used to capture SEA (*SEA-LDN*). One representative experiment of three is shown. *D*, the binding of fluorescent beads coated with biotinylated SEA (SEA-bio) was blocked by AZN-D2 (20 μg/ml) and EGTA (5 mM). One representative experiment of three is shown.

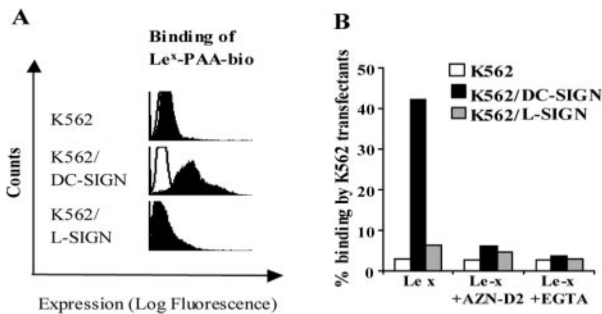

FIG. 2. **Binding of Le^x coupled to biotinylated polyacrylamide (Le^x-PAA-bio) to K562 cells expressing L-SIGN or DC-SIGN.** *A*, cells were incubated with Le^x-PAA-bio in adhesion buffer for 30 min at 37 °C. After incubation with goat-anti-mouse streptavidin Alexa Fluor488, the binding was measured by FACScan analysis, and shown as a histoplot. The *white graphs* represent control cells, the *black graphs* show binding of the cells to Le^x-PAA-bio. One representative experiment of three is shown. *B*, binding of K562 cells expressing L-SIGN or DC-SIGN to Le^x-PAA-bio (indicated as Le^x) was measured as outlined in *A* but shown here as percent binding. Binding of Le^x-PAA-bio to DC-SIGN could be blocked by EGTA and the anti-L-SIGN/DC-SIGN antibody AZN-D2. One representative experiment of three is shown.

L-SIGN mutant forms was compared with K562 cells expressing similar levels of wild-type L-SIGN, DC-SIGN, and the DC-SIGN V351G mutant, respectively (Fig. 3*B*). Both wild-type L-SIGN and DC-SIGN showed binding to biotinylated neoglycoconjugates containing Le^a, Le^b, and Le^y but not to sialyl Le^x (sLe^x) (Table I and Fig. 3*C*). EGTA and a blocking anti-DC-SIGN/L-SIGN antibody could inhibit this binding (data not shown). Remarkably, the L-SIGN mutant S363V showed binding to Le^x, in contrast to the wild-type L-SIGN (Fig. 3*C*). The introduction of this Val residue also induced increased binding of the L-SIGN S363V mutant to Le^a, Le^b, and Le^y (Fig. 3*C*), and to *S. mansoni* SEA (data not shown). The conversion of Val^{351} into a Gly in DC-SIGN (DC-SIGN V351G) abrogated binding to all Lewis antigens. Similarly, the L-SIGN S363G mutant showed no binding to any of the Lewis antigens tested (Fig. 3*C*). These results demonstrate that cell-surface-expressed L-SIGN and DC-SIGN differ in their binding properties to Le^x. Both lectins, however, show a functional similarity in their capacity to bind to Le^a, Le^b, and Le^y glycan antigens, and their lack of binding to sLe^x. Our data indicate that amino acid residue Val^{351} in DC-SIGN, which is lacking in L-SIGN, is critically involved in the binding of DC-SIGN to Le^x glycan structures.

Docking of Lewis x in DC-SIGN—To gain more insight in the molecular basis that determines the differences in binding properties of DC-SIGN and L-SIGN to Le^x, molecular modeling studies were undertaken. Because DC-SIGN and L-SIGN have been crystallized with mannose-containing oligosaccharides (17), but not with fucose containing ones, the possible interactions of Le^x with the CRD of DC-SIGN was determined first. Several binding modes of fucose, or fucose-containing oligosaccharides, have been observed when comparing crystal struc-

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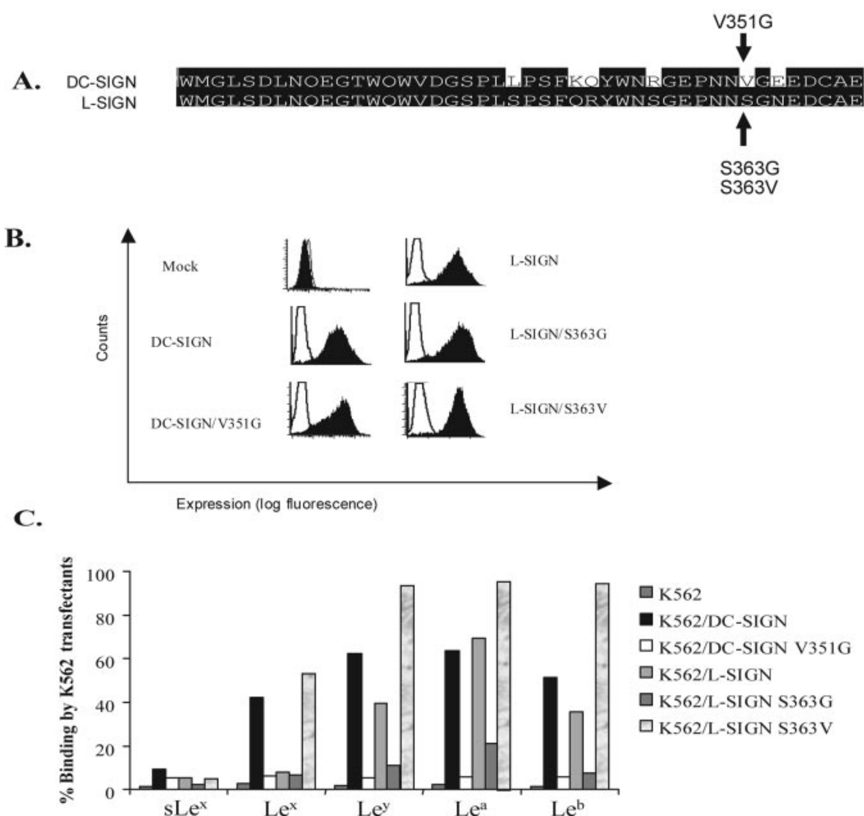

FIG. 3. **Binding of different Lewis antigens coupled to PAA-bio to K562 cells expressing L-SIGN, DC-SIGN, or mutant forms of the lectins.** *A*, amino acid sequence alignment of part of the CRDs of L-SIGN (AAK20998) and DC-SIGN (AAK20997) is depicted. The CRD sequences are very similar as is shown by the *black boxes*. Mutations in the CRD of L-SIGN have been introduced at position Ser^{363} as indicated by an *arrow*. *B*, stable K562 transfectants express similar levels of L-SIGN, DC-SIGN and different mutant forms of L-SIGN and DC-SIGN, as determined by FACScan analysis using monoclonal antibody AZN-D2. One representative experiment of three is shown. *C*, binding of different Lewis antigens coupled to PAA-bio to K562 cells expressing wild-type or mutant forms of L-SIGN and DC-SIGN. Cells were incubated with the PAA-bio-neoglycoconjugates in adhesion buffer for 30 min at 37 °C. After incubation with goat-anti-mouse streptavidin-Alexa Fluor 488, the binding was measured by FACScan analysis as described in the legend to Fig. 2 and shown as percent binding. One representative experiment of three is shown.

TABLE I
Structures of Lewis glycan antigens

Lewis antigen	Carbohydrate structure
Lewis x (Le^x)	Galβ1→4GlcNAc-R 3 ↑ Fucα1
Lewis y (Le^y)	Fucα1→2Galβ1→4GlcNAc-R 3 ↑ Fucα1
Lewis a (Le^a)	Galβ1→3GlcNAc-R 4 ↑ Fucα1
Lewis b (Le^b)	Fucα1→2Galβ1→3GlcNAc-R 4 ↑ Fucα1
sialyl-Lewis x (sLe^x)	NeuAcα1→3Galβ1→4GlcNAc-R 3 ↑ Fucα1

tures of the whole C-type lectin family. sLe^x is bound to E- and P-selectin with oxygen O-3 and O-4 of fucose interacting with the calcium ion (33). Alternatively, fucose and several sialyl and sulfo-Le^x derivatives are bound to mannose-binding protein with O-2 and O-3 of fucose involved in calcium coordination (34–36).

Four docking modes were tested in the present study for Le^x interaction with DC-SIGN, the two described above and two additional ones, with an inversion of the fucose orientation (*i.e.* inversion of O-4 and O-3 in the first binding mode and inversion of O-2 and O-3 in the second one). Only the two binding modes previously observed in C-type lectins yielded stable interactions without steric or hydrophobic conflict (Fig. 4). Binding mode A, which corresponds to the interaction observed for sLe^x in E- and P-selectin, is energetically favored, because it is stabilized by five hydrogen bonds between the fucose and the protein and an additional one between O-6 of galactose and an acidic group (Fig. 5). This particular hydrogen bond is also observed in selectins with a conserved glutamate residue (33). The second binding mode (Fig. 4*B*), corresponding to sulfo and sLe^x in mannose-binding protein mutants (35), only displays four hydrogen bonds. Thus it is proposed that DC-SIGN and L-SIGN bind fucosylated oligosaccharides with similar orientations as found in the selectins (Fig. 4*A*).

Docking of Other Oligosaccharides in DC-SIGN—The energy-minimized structures of DC-SIGN in complex with Le^x and Le^a trisaccharides and Le^y and Le^b tetrasaccharides have been calculated. The six hydrogen bonds described above are conserved in all of these complexes (Table II). In addition, hydrophobic contact can be predicted between galactose aliphatic groups and the aromatic ring of the amino acid residue Phe^{313}. Val^{351} of DC-SIGN is close to the fucose binding site and makes strong hydrophobic contact with CH at position 1 and 2 of fucose. For Le^x, the methyl group of GlcNAc is also involved in this hydrophobic patch (Fig. 5*A*), whereas for Le^a, the CH_2 of the hydroxymethyl group plays the same role albeit at slightly longer distance (Fig. 5*B*). No additional contacts are observed for Le^b and Le^y. This model is fully compatible with the observed binding of DC-SIGN to all Lewis antigens. In addition, sLe^x has been docked in the binding site of DC-SIGN with the same conformation as observed in E- and P-selectin crystals (33). In the latter structures, the acidic group of NeuAc closely interacts with amino acid residue Tyr^{48} allowing for the occurrence of a strong hydrogen bond between the acidic group of

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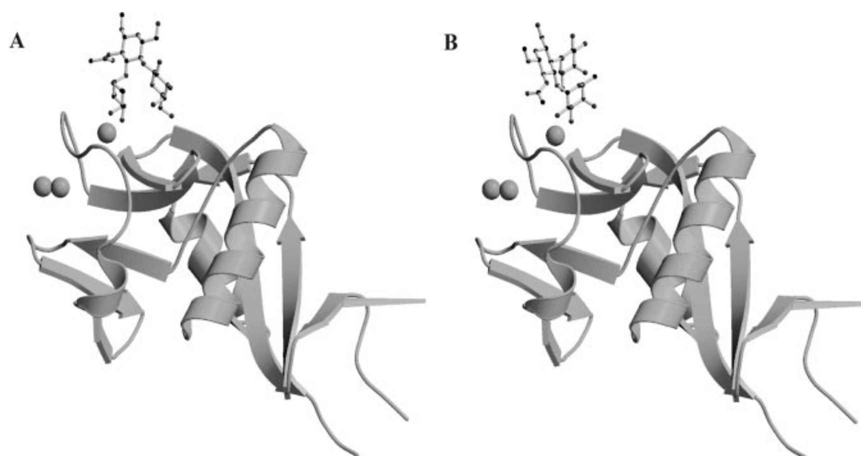

FIG. 4. **Two proposed binding modes for Le^x trisaccharide with DC-SIGN.** Ribbon presentation of the binding modes of Le^x trisaccharide to DC-SIGN. The bound calcium ions in the structure are represented as *gray spheres*. *A*, similar to the observed binding of sialyl-Le^x in E-selectin (33). *B*, similar to the observed binding site of 3-sulfo-Lewis x in modified mannose-binding protein-A (35). All drawings were performed with the MOLSCRIPT program (37).

NeuAc monosaccharide and the hydroxyl group of the Tyr. However, in DC-SIGN the equivalent position is occupied by Phe^{313}. There is no favorable interaction between the acidic group of NeuAc and the hydrophobic aromatic group of Phe^{313}, which may explain the lack of binding of DC-SIGN to sLe^x.

Docking of Oligosaccharides in L-SIGN—The hydrophilic amino acids of the L-SIGN binding site are identical to the ones in DC-SIGN, and the Lewis oligosaccharides can be docked with establishing the same network of six hydrogen bonds (Table II). The only difference is Ser^{363} that replaces Val^{351} in DC-SIGN. The substitution of a Val by a Ser destroys the hydrophobic wall that was adjacent to the fucose residue and creates a hydrophilic pocket that is not favorable for the methyl group of GlcNAc in Le^x (Fig. 5*C*). This may explain the lack of binding of L-SIGN to Le^x. However, when Le^a is docked, Ser^{363} can establish a hydrogen bond with the O-6 of GlcNAc thereby restoring part of the contacts that are lost (Fig. 5*D*) resulting in binding of Le^a. Le^b and Le^y have been modeled and did not present additional interactions compared with their corresponding trisaccharides Le^a and Le^x, respectively (data not shown). Although this model explains binding of L-SIGN to Le^b, the observed binding of L-SIGN to Le^y is not supported by the model proposed.

Docking of Oligosaccharides in Mutant Forms of L-SIGN and DC-SIGN—When Val^{351} of DC-SIGN is substituted by a Gly residue, the hydrophobic interaction with the fucose residue is lost. Furthermore, the GlcNAc residue does not interact at all with the protein surface in this mutant, neither for Le^x nor for Le^a (Fig. 5, *E* and *F*). The loop that contains this Gly appears to be stable in our calculations, but it could have greater mobility in solution, which will be entropically unfavorable for the binding of oligosaccharides. The same observations are made for the S363G mutant in L-SIGN (data not shown). These data are compatible with the observed lack of binding of both the DC-SIGN V351G and L-SIGN S363G mutant to all Lewis antigens. By contrast, the L-SIGN S363V mutant displays the same strong hydrophobic stabilization of the fucose and the methyl (or hydroxymethyl group) of GlcNAc as is observed in DC-SIGN (data not shown), which corresponds with the observed increase in binding of this mutant to all Lewis antigens and *S. mansoni* SEA.

DISCUSSION

L-SIGN and DC-SIGN contain a single CRD, which mediates recognition of either self-glycoproteins or carbohydrate antigens on pathogens (38, 39). The CRDs of L-SIGN and DC-SIGN show a high amino acid sequence identity suggesting that their carbohydrate recognition profiles may be similar. Recently we have demonstrated that DC-SIGN binds to *S. mansoni* SEA through recognition of Le^x antigens (15). Here we show that L-SIGN binds to a different subset of SEA than DC-SIGN and does not bind Le^x antigens. These data show, for the first time, a clear difference in binding properties between L-SIGN and DC-SIGN, which may have important consequences for their functions. In *S. mansoni* infections, egg antigens and their major glycan antigen Le^x are able to cause a switch toward Th2-mediated immune responses (40). Although direct evidence is still lacking, we consider it possible that the interaction between Le^x and DC-SIGN may contribute to a shift in the Th1/Th2 balance in favor of persistence of the pathogen. The binding of L-SIGN to SEA suggests that also this C-type lectin could play an important role in the recognition of *S. mansoni* egg antigens. However, the specific antigens within SEA that are involved in interaction with L-SIGN as well as the functional relevance of this interaction need to be further investigated.

Our previous studies (15) suggested that amino acid residue Val^{351} in DC-SIGN may have a crucial role in binding the Le^x antigen. Here we show that the mutation of Val^{351} into Gly in DC-SIGN not only abrogated binding of DC-SIGN to Le^x but also to Le^a, Le^b, and Le^y glycan antigens. L-SIGN has Ser^{363} in the position equivalent to Val^{351} of DC-SIGN. Remarkably, although L-SIGN does not bind to Le^x, it shows binding to other Lewis antigens. Docking of the Lewis oligosaccharides into molecular models of the CRDs of L-SIGN and DC-SIGN indicated that the Lewis antigens most likely dock in a mode similar to sLe^x in E-selectin (Fig. 4). Apart from being energetically the most favorable mode, only this mode is in agreement with the data demonstrating that DC-SIGN and L-SIGN do not bind sLe^x. In the docking mode based on mannose-binding protein-A, sialic acid does not make any additional contacts compared with Le^x, which would predict binding of DC-SIGN and L-SIGN to both Le^x and sLe^x and contradicts binding studies.

From the proposed models it appears that Val^{351} in DC-SIGN is close to the fucose binding site and makes a strong hydrophobic contact with CH at position 1 and 2 of fucose. In L-SIGN the presence of a Ser instead of a Val creates a hydrophilic pocket that is not favorable for the methyl group of GlcNAc in Le^x but can establish a hydrogen bond with the O-6 of GlcNAc. This may explain the observed differences of L-SIGN binding to Le^x and Le^a. However, changing L-SIGN Ser^{363} into a Val not only allowed binding to Le^x but also increased binding to all Lewis antigens, showing that a Val residue is favored for binding the fucose-containing oligosaccharides in the CRD domain.

All binding assays in this study have been performed with cells expressing the recombinant C-type lectins or their mutant forms at the cell surface where they may be assembled into

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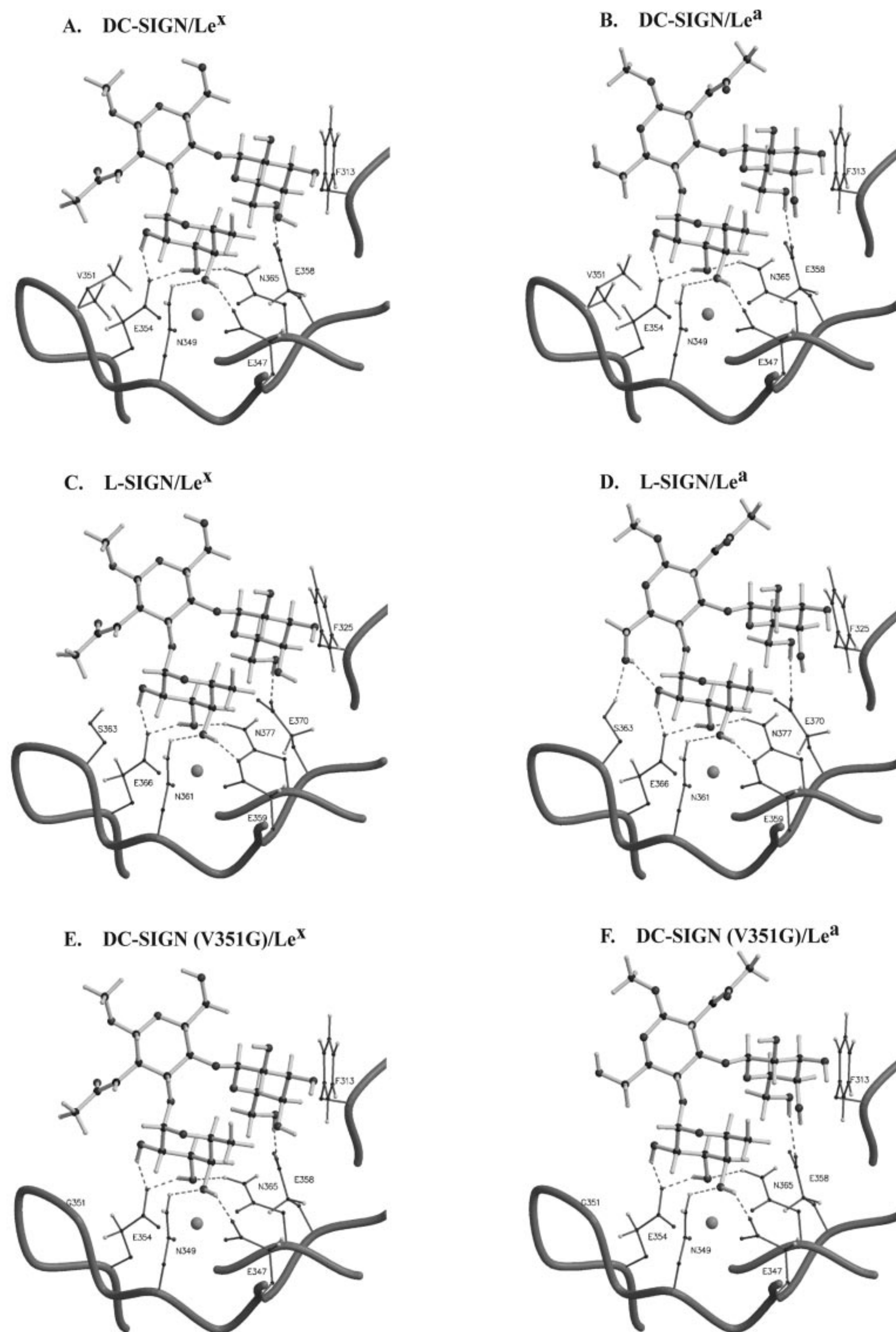

FIG. 5. **Models of the interaction of DC-SIGN, L-SIGN, and DC-SIGN/V351G with Le^x and Le^a.** Models of the interaction of DC-SIGN with Le^x (*A*) and Le^a trisaccharides (*B*), L-SIGN with Le^x (*C*) and Le^a trisaccharides (*D*), and DC-SIGN (V351G) with Le^x (*E*) and Le^a trisaccharides (*F*). The calcium ion is represented by a *gray sphere*. Only the amino acids interacting directly with the sugars have been displayed.

tetramers and interact with glycan ligands presented in multivalent form (16). The models that show docking of Le^x, Le^a, Le^b, and sLe^x in the CRDs of DC-SIGN, L-SIGN, and their mutant forms correspond very well to the observed results of the binding assays. However, comparison of the docking of Le^y with Le^x in L-SIGN did not reveal additional interactions that would explain binding of Le^y, but not Le^x, by L-SIGN. It is possible that either additional contacts between Le^y and the CRD of L-SIGN are introduced in the tetrameric form of L-SIGN or that more than one CRD is involved in the binding of the oligosaccharide, which may result in another binding mode of Le^y in L-SIGN than proposed from the model.

It is remarkable, that DC-SIGN and L-SIGN interact with such different oligosaccharide ligands, *i.e.* high mannose-type *N*-glycans and Lewis antigens using the same region in their CRDs. Many of the amino acid residues that interact with the mannose-type glycans, *i.e.* Phe^{313}, Glu^{347}, Asn^{349}, Val^{351}, Glu^{354}, and Asn^{365} in DC-SIGN, and their corresponding residues in L-SIGN (16) are proposed here to be also involved in binding to the Lewis structures. Interestingly, these amino acid residues clearly interact with different monosaccharides, and the individual importance of each of these contacts in the

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TABLE II
Hydrogen bonds involved in the binding of Lewis oligosaccharides by DC-SIGN and L-SIGN

Sugar atom	Donor/accept	DC-SIGN	L-SIGN
H-bonds predicted for all Lewis oligosaccharides			
Fuc O-2	→	Glu^{354} OE-2	Glu^{366} OE-2
Fuc O-3	→	Glu^{354} OE-2	Glu^{366} OE-2
Fuc O-3	←	Asn^{365} ND-2	Asn^{377} ND-2
Fuc O-4	→	Glu^{347} OE-2	Glu^{359} OE-2
Fuc O-4	←	Asn^{349} ND-2	Asn^{361} ND-2
Gal O-6	→	Glu^{358} OE-1	Glu^{370} OE-1
H-bond predicted for Lewis a and Lewis b oligosaccharides			
GlcNAc O-6	←		Ser^{363} OG

binding pocket may vary depending on the glycan that is bound. Recently it was shown that mutating Gly^{346} in DC-SIGN abrogated gp120 binding but enhanced ICAM-2 and ICAM-3 binding (41), whereas mutation of Val^{351} abrogates binding to ICAM-3 and Le^{x} but not to HIV-1 gp120 (15, 28). Thus, DC-SIGN appears to bind in a distinct but overlapping manner to gp120 when compared with ICAM-2, ICAM-3, and Le^{x}. Differential recognition of carbohydrate ligands by DC-SIGN and L-SIGN will be crucially involved in the functional consequences of these interactions, which may lead to either immune activation or suppression. Detailed structural knowledge of the molecular interactions of DC-SIGN and L-SIGN with their carbohydrate ligands may be exploited to develop strategies for immune intervention, such as HIV-1 dissemination by DC-SIGN or dendritic cell-induced immunity.

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