DC-SIGN Family of Receptors

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36.1 DC-SIGN (CD209) Family of Receptors

In the immune system, C-type lectins and CTLDs have been shown to act both as adhesion and as pathogen recognition receptors. The **D**endritic **c**ell-**s**pecific **I**CAM-3 **g**rabbing **n**on-integrin (DC-SIGN) and its homologs in human and mouse represent an important C-type lectin family. DC-SIGN contains a lectin domain that recognizes in a Ca^{2+}-dependent manner carbohydrates such as mannose-containing structures present on glycoproteins such as ICAM-2 and ICAM-3. DC-SIGN is a prototype C-type lectin organized in microdomains, which have their role as pathogen recognition receptors in sensing microbes. Although the integrin LFA-1 is a counter-receptor for both ICAM-2 and ICAM-3 on DC, DC-SIGN is the high affinity adhesion receptor for ICAM-2/-3. While cell–cell contact is a primary function of selectins, collectins are specialized in recognition of pathogens. Interestingly, DC-SIGN is a cell adhesion receptor as well as a pathogen recognition receptor. As adhesion receptor, DC-SIGN mediates the contact between dendritic cells (DCs) and T lymphocytes, by binding to ICAM-3, and mediates rolling of DCs on endothelium, by interacting with ICAM-2. As pathogen receptor, DC-SIGN recognizes a variety of microorganisms, including viruses, bacteria, fungi and several parasites (Cambi et al. 2005). The natural ligands of DC-SIGN consist of mannose oligosaccharides or fucose-containing Lewis-type determinants. In this chapter, we shall focus on the structure and functions of DC-SIGN and related CTLDs in the recognition of pathogens, the molecular and structural determinants that regulate the interaction with pathogen-associated molecular patterns. The heterogeneity of carbohydrate residues exposed on cellular proteins and pathogens regulates specific binding of DC-expressed C-type lectins that contribute to the diversity of immune responses created by DCs (van Kooyk et al. 2003a; Cambi et al. 2005).

The DC-SIGN, originally described in 1992 as a C-type lectin able to bind the HIV surface protein, gp120 (Curtis et al. 1992), is important for efficient infection with HIV (Geijtenbeek et al. 2000c). Recent advances on a broad perspective concerning DC-SIGN structure, signaling and immune function have appeared in excellent reviews (den Dunnen et al. 2009; Gringhuis and Geijtenbeek 2010; Svajger et al. 2010). Interaction of DC-SIGN with the viral envelope glycoproteins may evoke cellular signal transduction implicated in viral pathogenesis.

36.1.1 CD209 Family Genes in Sub-Human Primates

Two *CD209* family genes identified in humans, *CD209* (*DC-SIGN*) and *CD209L* (*DC-SIGNR*/*L-SIGN*/*LSECtin*), encode C-type lectins that serve as adhesion receptors for ICAM-2 and ICAM-3 and participate in the transmission of HIV and SIV respectively to target cells in vitro. The *CD209* gene family that encodes C-type lectins in primates includes CD209 (DC-SIGN), CD209L (L-SIGN) and CD209L2. The *CD209* gene family in sub-human primates showed evolutionary alterations that occurred in this family across primate species. All of the primate species, specifically, Old World monkeys (OWM) and apes, have orthologues of human *CD209*. In contrast, *CD209L* is missing in OWM but present in apes. A third family member, that has been named *CD209L2*, was cloned from rhesus monkey cDNA and subsequently identified in OWM and apes but not in humans. Rhesus *CD209L2* mRNA was prominently expressed in the liver and axillary lymph nodes. Despite a high level of sequence similarity to both human and rhesus CD209, rhesus CD209L2 was substantially less effective at binding ICAM-3 and poorly transmitted HIV type 1 and SIV to target cells relative to CD209. The Toll-like receptor (TLR) gene family shares with CD209 genes a common

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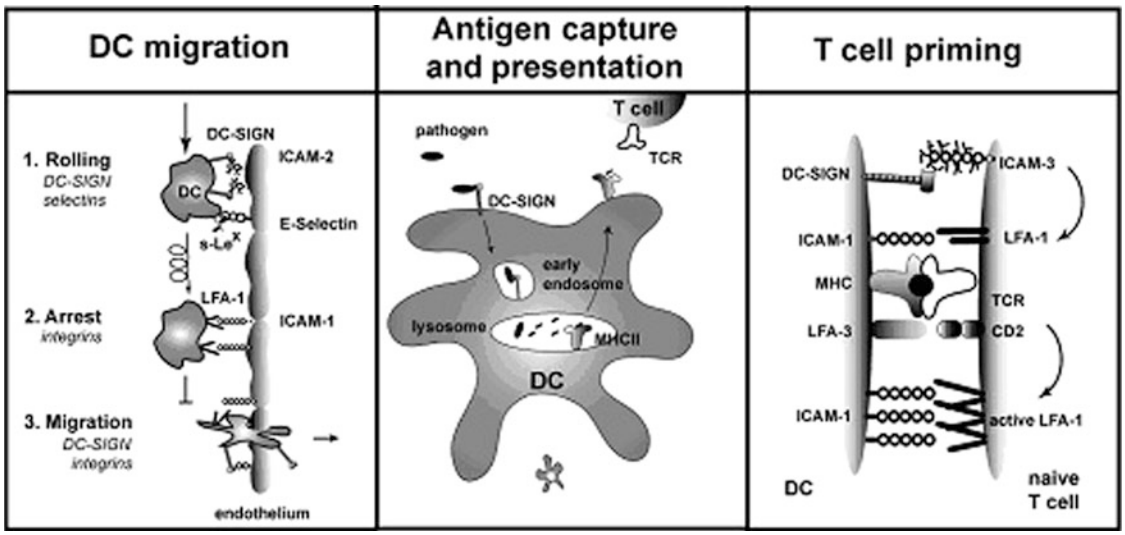

Fig. 36.1 DC-SIGN controls many functions of DC to elicit immune responses. The egress of precursor DC from blood into tissues is mediated partly by DC-SIGN. DC-SIGN facilitates rolling and transendothelial migration of DC-SIGN_ precursor DC, whereas arrest is mediated by integrin-mediated interactions. DC-SIGN also functions as an antigen receptor. DC-SIGN internalizes rapidly upon binding soluble ligand and is targeted to late endosomes/lysosomes, where antigens are processed and presented by MHC class II molecules. Moreover, initial DC-T-cell clustering, necessary for an efficent immune response, is mediated by transient interactions between DC-SIGN and ICAM-3. This interaction facilitates the formation of low-avidity LFA-1/ICAM-1 interaction and scanning of the antigen-MHC repertoire. It is becoming clear that other C-type lectins also participate in these processes. Selectins and the MR may regulate DC migration. Many other C-type lectins on DC, such as MR, DEC205, DC-ASPGR, BCDA-2, and dectin-1, are antigen receptors that recognize various distinct carbohydrate-containing antigens. It has been postulated that dectin-1 regulates T-cell priming, however its interaction with T cells is not carbohydrate-dependent (Reprinted with permission from Geijtenbeek et al. 2002a © Journal of Leukocyte Biology)

profile of evolutionary constraint (Bashirov et al. 2003b; Ortiz et al. 2008).

36.2 DC-SIGN (CD209): An Adhesion Molecule on Dendritic Cells

DC-SIGN (dendritic cell-specific ICAM-grabbing non-integrin, where ICAM is intercellular adhesion molecule) or CD209 is a type II C-type lectin expressed by DCs. DC-SIGN is specifically expressed on DCs and has been identified on monocyte-derived DCs in vitro and on DC subsets of skin, mucosal tissues, tonsils, lymph nodes, and spleen in vivo (Geijtenbeek et al. 2000a, c). DC-SIGN on DC binds the intercellular adhesion molecule (ICAM)-3 (CD50) with very high affinity. Although ICAM-3, a member of the immunoglobulin (Ig) superfamily, is known to be a ligand for β2 integrins lymphocyte function-associated antigen-1 (LFA-1; αLβ2) and $\alpha_D\beta_2$ (Bleijs et al. 2001; Geijtenbeek et al. 2002a; van Kooyk and Geijtenbeek 2002) (Fig. 36.1), these receptors did not contribute to the binding activity of ICAM-3 by DC. Many C-type lectins have been identified on DCs: (1) Type I multi-CRD lectins are represented by the mannose receptor (Sallusto et al. 1995) and DEC-205 (Kato et al. 2000); and (2) type II single-CRD lectins by DC-SIGN, dectin-1, dectin-2, Langerin, BCDA-2, DCIR, DLEC, CLEC-1, and DC-ASGPR (Geijtenbeek et al. 2000a) (Chap. 35). Within the CRD, the highly conserved EPN or QPD sequences are essential in recognizing mannose- and galactose-containing structures, respectively.

DC-SIGN contains a short, cytoplasmic N-terminal domain with several intracellular sorting motifs, an extracellular stalk of seven complete and one partial tandem repeat, and a terminal lectin or CRD (Geijtenbeek et al. 2000a; Curtis et al. 1992). The DC-SIGN has three conserved cytoplasmic tail motifs: the tyrosine (Y)-based, dileucine (LL), and triacidic cluster (EEE), which are believed to regulate ligand binding, uptake, and trafficking. The full-length porcine DC-SIGN cDNA encodes a type II transmembrane protein of 240 amino acids. Phylogenetic analysis revealed that porcine DC-SIGN, together with bovine, canis and equine DC-SIGN, is more closely related to mouse SIGNR7 and SIGNR8 than to human DC-SIGN. Porcine DC-SIGN has the same gene structure as bovine, canis DC-SIGN and mouse SIGNR8 with eight exons. Porcine DC-SIGN mRNA expression was detected in spleen, thymus, lymph node, lung, bone marrow and muscles. Porcine DC-SIGN protein was found to express on the surface of monocyte-derived macrophages and dendritic cells, alveolar macrophages, lymph node sinusoidal macrophage-like, dendritic-like and endothelial cells but not of monocytes, peripheral blood lymphocytes or lymph node lymphocytes (Huang et al. 2009). Bovine ortholog of human DC-SIGN,

within the bovine genome, exists as a single copy with a sequence similar to that of SIGNR7 (Yamakawa et al. 2008).

36.3 Ligands of DC-SIGN

36.3.1 Carbohydrates as Ligands of DC-SIGN

DC-SIGN and its close relative DC-SIGNR recognize various oligosaccharide ligands found on human tissues as well as on pathogens including viruses, bacteria, and parasites through the receptor lectin domain-mediated carbohydrate recognition. The DC-SIGN and DC-SIGNR bind to high-mannose carbohydrates on a variety of viruses. Studies have shown that these receptors bind the outer trimannose branch Manα1-3(Manα1-6)Manα present in high mannose structures. Although the trimannoside binds to DC-SIGN or DC-SIGNR more strongly than mannose, additional affinity enhancements are observed in presence of one or more Manα1-2Manα moieties on nonreducing termini of oligomannose structures. The molecular basis of this enhancement was investigated in crytstals of DC-SIGN bound to a synthetic six-mannose fragment of a high mannose N-linked oligosaccharide, Manα1-2Manα1-3 (Manα1-2Manα1-6)Manα1-6Man and to the disaccharide Manα1-2Man. The structures revealed mixtures of two binding modes in each case. Each mode features typical C-type lectin binding at main Ca^{2+}-binding site by one mannose residue. In addition, other sugar residues form contacts unique to each binding mode. Thus the affinity enhancement of DC-SIGN toward oligosaccharides decorated with Manα1-2Manα structure is due to multiple binding modes at the primary Ca^{2+} site, which provides both additional contacts and a statistical (entropic) enhancement of binding (Feinberg et al. 2007).

36.3.1.1 Lewis Antigen as Ligand

In addition to high-mannose moieties, DC-SIGN recognizes nonsialylated $Lewis^X$ (Le^x) and Le^Y glycans and binds to Le^X-expressing pathogens such as *Schistosoma mansoni* and *Helicobacter pylori* (Appelmelk et al. 2003; van Die et al. 2003). Mouse homolog of human DC-SIGN has similar carbohydrate specificity for high mannose-containing ligands present on both cellular and pathogen ligands and called mSIGNR1or SIGNR1. However, mSIGNR1 interacts not only with $Le^{x/y}$ and $Le^{a/b}$ antigens similar to DC-SIGN, but also with sialylated Le^x, a ligand for selectins. The differential recognition of Lewis antigens suggests differences between mSIGNR1 and DC-SIGN in the recognition of cellular ligands and pathogens that express Lewis epitopes (Koppel et al. 2005a). Using the known 3D structure of the Lewis-x trisaccharide, Timpano et al. (2008) identified some monovalent α-fucosylamides that bind to DC-SIGN. α-fucosylamides work as functional mimics of chemically and enzymatically unstable α-fucosides and describes interesting candidates for the preparation of multivalent systems able to block DC-SIGN with high affinity and with potential biomedical applications.

The binding partner of DC-SIGN on endothelial cells is the glycan epitope Le^Y, expressed on ICAM-2. The interaction between DC-SIGN on DCs and ICAM-2 on endothelial cells is strictly glycan-specific. ICAM-2 expressed on CHO cells only served as a ligand for DC-SIGN when properly glycosylated, underscoring its function as a scaffolding protein (García-Vallejo et al. 2008). Oligosaccharide ligands expressed on SW1116, a typical human colorectal carcinoma are recognized by DC-SIGN, and has similar carbohydrate-recognition specificities as MBP. These tumor-specific oligosaccharide ligands comprise clusters of tandem repeats of Le^a/Le^b glycans on carcinoembryonic Ag (CEA) and CEA-related cell adhesion molecule 1 (CEACAM1). DC-SIGN ligands containing Le^a/Le^b glycans are also highly expressed on primary cancer colon epithelia but not on normal colon epithelia (Nonaka et al. 2008). Fucosylated glycans similar to pathogens are also found in a variety of allergens, but their functional significance remains unclear. Results suggest that allergens are able to interact with DC-SIGN and induce TNF-α expression in monocyte-derived DCs (MDDCs) via, in part, Raf-1 signaling pathways (Hsu et al. 2010).

Structural characterization of glycolipids, in combination with solid phase and cellular binding studies revealed that DC-SIGN binds to carbohydrate moieties of both glycosphingolipid species with Galβ1-4(Fucα1-3)GlcNAc (Le^x) and Fucα1-3Galβ1-4(Fucα1-3)GlcNAc (pseudo- Le^y) determinants. These data indicate that surveying DCs in the skin may encounter schistosome-derived glycolipids immediately after infection. Crystal structure of the CRD of DC-SIGN bound to Le^x provided insight into the ability of DC-SIGN to bind fucosylated ligands. The observed binding of schistosome-specific pseudo-Le^y to DC-SIGN is not directly compatible with the model described (Meyer et al. 2005).

36.4 Structure of DC-SIGN

36.4.1 Neck-Domains

Human DC-SIGN is a type II membrane protein which contains 404 amino acids and is of 44 kDa in molecular weight. DC-SIGN consists of extracellular domain, transmembrane region and cytoplasmic region (Fig. 36.2). The extracellular portion of each receptor contains a membrane-distal CRD and forms tetramers stabilized by an extended neck region consisting of 23 amino acid repeats. Cross-linking analysis of full-length receptors expressed in

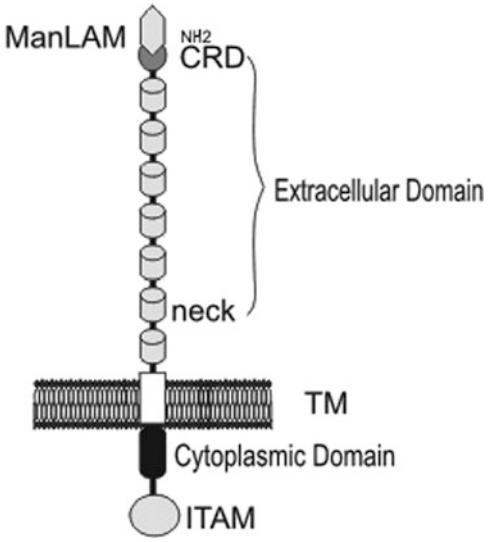

Fig. 36.2 Structure of DC-SIGN. Cytoplasmic domain, transmembrane region (TM) and extracellular domain are the three parts of DC-SIGN. The extracellular domain contains carbohydrate recognition domain (CRD) and neck domain. Cytoplasmic domain contains LL (di-leucine), EEE (tri-acidic clusters) and other internalization motifs and is connected to an incomplete ITAM. CRD recognizes certain carbohydrate-contained antigens like ManLAM and $Lewis^X$ by four amino acids (Glu^{347}, Asn^{349}, Glu^{354} and Asn^{365}) and one Ca^{2+}-binding site in it (Zhou et al. 2006)

fibroblasts confirmed the tetrameric state of the intact receptors. Alternative splicing and genomic polymorphism generate DC-SIGN mRNA variants, which were detected at the sites of pathogen entrance and transmission. Naturally occurring DC-SIGN neck variants differ in multimerization competence in the cell membrane, exhibit altered sugar binding ability, and retain pathogen-interacting capacity, implying that pathogen-induced cluster formation predominates over the basal multimerization capability. Reports highlight the central role of the neck domain in the pH-sensitive control of oligomerization state, in the extended conformation of the protein, and in CRD organization and presentation (Guo et al. 2006; Tabarani et al. 2009). Analysis of DC-SIGN neck polymorphisms indicated that the number of allelic variants is higher than previously thought and that the multimerization of the prototypic molecule is modulated in presence of allelic variants with a different neck structure. Serrano-Gómez et al. (2008) demonstrated that the presence of allelic variants or a high level of expression of neck domain splicing isoforms might influence the presence and stability of DC-SIGN multimers on the cell surface, thus providing a molecular explanation for the correlation between DC-SIGN polymorphisms and altered susceptibility to HIV-1 and other pathogens. Neck domains of DC-SIGN and DC-SIGNR are shown to form tetramers in the absence of the CRDs. Analysis indicates that interactions between the neck domains account full stability of the tetrameric extracellular portions of the receptors. The neck domains are ~40% α-helical based on circular dichroism analysis. However, in contrast to other glycan-binding receptors in which fully helical neck regions are intimately associated with C-terminal C-type CRDs, the neck domains in DC-SIGN and DC-SIGNR act as autonomous tetramerization domains and the neck domains and CRDs are organized independently. Neck domains from polymorphic forms of DC-SIGNR that lack some of the repeat sequences show modestly reduced stability, but differences near the C-terminal end of the neck domains lead to significantly enhanced stability of DC-SIGNR tetramers compared to DC-SIGN (Yu et al. 2009). The length of the neck region shows variable levels of polymorphism, and can critically influence the pathogen binding properties of these two receptors. In Colored South African population of 711 individuals, including 351 tuberculosis patients and 360 healthy controls, Barreiro et al. (2007) revealed that none of the DC-SIGN and L-SIGN neck-region variants or genotypes seems to influence the individual susceptibility to develop tuberculosis.

Surface force measurements between apposed lipid bilayers displaying the extracellular domain of DC-SIGN and a neoglycolipid bearing an oligosaccharide ligand provide evidence that the receptor is in an extended conformation and that glycan docking is associated with a conformational change that repositions the carbohydrate-recognition domains during ligand binding. The results further show that the lateral mobility of membrane-bound ligands enhances the engagement of multiple carbohydrate-recognition domains in the receptor oligomer with appropriately spaced ligands. These studies highlight differences between pathogen targeting by DC-SIGN and receptors in which binding sites at fixed spacing bind to simple molecular patterns (Menon et al. 2009).

36.4.2 Crystal Structure of DC-SIGN (CD209) and DC-SIGNR (CD299)

To understand the tetramer-based ligand binding avidity, the crystal structure of DC-SIGNR was determined with its last repeat region. Compared to the carbohydrate-bound CRD structure, the structure revealed conformational changes in the calcium and carbohydrate coordination loops of CRD, an additional disulfide bond between the N and the C termini of the CRD, and a helical conformation for the last repeat. On the basis of the current crystal structure and other published structures with sequence homology to the repeat domain, Snyder et al. (2005) generated a tetramer model for DC-SIGN/DC-SIGNR using homology modeling and proposed a ligand-recognition index to identify potential receptor ligands.

The CRD of DC-SIGN is a globular structure consisting of 2 α-helices, 12 β-strands, and 3 disulphide bridges. A loop protrudes from the protein surface and forms part of two Ca^{2+}- binding sites. One of such sites is essential for the conformation of CRD, and the other is essential for direct coordination of the carbohydrate structures. Four amino acids (Glu^{347}, Asn^{349}, Glu^{354} and Asn^{365}) interact with Ca^{2+} at this site and dictate the recognition of specific carbohydrate structures. CRD can recognize certain carbohydrate containing antigens like ManLAM and Le^{x}. Crystal structures of CRDs of DC-SIGN and of DC-SIGNR bound to oligosaccharide revealed that these receptors selectively recognize endogenous high-mannose oligosaccharides and represent a new avenue for developing HIV prophylactics (Feinberg et al. 2001). Hydrodynamic studies on truncated receptors demonstrated that the portion of the neck of each protein adjacent to the CRD was sufficient to mediate the formation of dimers, whereas regions near the N terminus were needed to stabilize the tetramers. Some of the intervening repeats are missing from polymorphic forms of DC-SIGNR. Two different crystal forms of truncated DC-SIGNR comprising two neck repeats and the CRD revealed that the CRDs are flexibly linked to the neck, which contains α-helical segments interspersed with non-helical regions. Differential scanning calorimetry measurements indicated that the neck and CRDs were independently folded domains (Feinberg et al. 2005).

The neck domain contains 7 or 8 complete tandem repeats of 23 amino acids each and 1 incomplete repetitive sequence. It is required for oligomerization, which regulates carbohydrate specificity. Transmembrane region is essential in localization of DC-SIGN on cell surface. The cytoplasmic region contains internalization motifs, such as di-leucine (LL) motif, tri-acidic (EEE) clusters and an incomplete immunoreceptor tyrosine (Y) based activation motif (ITAM) which are believed to regulate ligand binding, uptake, and trafficking. The LL motif participates in antigen internalization and EEE clusters participate in signal transduction (Guo et al. 2004; Feinberg et al. 2001; van Kooyk and Geijtenbeek 2003). DC-SIGNR is homologous to DC-SIGN and is also denoted DC-related protein (L-SIGN), which has a similar structure to DC-SIGN. The genes encoding DC-SIGNR are similar to those of DC-SIGN (Pohlmann et al. 2001). Based on crystal structures and hydrodynamic data, models for the full extracellular domains of the receptors have been generated. The observed flexibility of the CRDs in the tetramer, combined with previous data on the specificity of these receptors, suggests an important role for oligomerization in the recognition of endogenous glycans, in particular those present on the surfaces of enveloped viruses recognized by these proteins (Feinberg et al. 2005). Azad et al. (2008) mutated each of the three conserved cytoplasmic tail motifs of DC-SIGN by alanine substitution and tested their roles in phagocytosis and receptor-mediated endocytosis of highly mannosylated ligands, *M. tuberculosis* ManLAM and HIV-1 surface gp120, respectively, in transfected human myeloid K-562 cells. Azad et al. (2008) indicated a dual role for EEE motif as a sorting signal in the secretory pathway and a lysosomal targeting signal in the endocytic pathway. The DC-SIGN and L-SIGN have been shown to interact with a vast range of infectious agents, including *M. tuberculosis*.

36.5 DC-SIGN versus DC-SIGN-RELATED RECEPTOR [DC-SIGNR or L-SIGN (CD 209)/LSEctin] (Refer Section 36.9)

36.5.1 DC-SIGN Similarities with DC-SIGNR/ L-SIGN/LSEctin

DC- SIGNR is homologous to DC-SIGN and is also denoted as DC- related protein (L-SIGN), which has a similar structure to DC-SIGN. The genes encoding DC-SIGNR are similar to those of DC-SIGN (Pohlmann et al. 2001). The mRNA of L-SIGN shows about 90% similarity with DC-SIGN, which has a similar binding specificity to L-SIGN. L-SIGN like DC-SIGN binds to the cellular ligands i.e. ICAM-2 and ICAM-3 (Bashirova et al. 2001). The sequences of CRDs of DC-SIGN and DC-SIGNR show greatest identity to human asialoglycoprotein receptors (41% and 34% at amino acid level, respectively) and rat CD23 (both 33% at amino acid level). Consistent with previous reports (Curtis et al. 1992; Geijtenbeek et al. 2000a, b), DC-SIGN shows features of a mannose binding lectin, as opposed to the features of a protein-binding NK cell lectin (Weis et al. 1998). DC-SIGNR shows 77% identity to DC-SIGN at the amino acid level and also possesses all the residues shown to be required for the binding of mannose (Weis et al. 1998). The closely linked gene, DC-SIGNR, shows 73% identity to DC-SIGN at RNA level and a similar genomic organization.

The DC-SIGN and DC-SIGNR/L-SIGN (or CLEC4M) directly recognize a wide range of micro-organisms of major impact on public health. Both genes have long been considered to share similar overall structure and ligand-binding characteristics. Both DC-SIGN and DC-SIGNR efficiently bind HIV-1 surface glycoproteins of viruses and other viral as well as nonviral pathogens by interacting with high mannose oligosaccharides and assist either cis or trans infection. DC-SIGNR/L-SIGN (Lozach et al. 2004) is specifically expressed by liver sinusoidal endothelial cells (LSEC), a liver-resident APC, by endothelial cells in lymph nodes (Bashirova et al. 2001; Pohlmann et al. 2001) and by placenta (Soilleux 2003b). Similar *trans* activity for other viruses has been reported, and the receptors can also directly mediate infection of cells in *cis* (Soilleux 2003; Alvarez

et al. 2002; Gardner et al. 2003). There is also evidence that these receptors interact with bacterial pathogens and with parasites (Appelmelk et al. 2003). The L-SIGN/DC-SIGNR functions as a HIV-1 trans-receptor similar to DC-SIGN (Bashirova et al. 2001). Moreover, L-SIGN interacts with other pathogens such as *Ebola virus* (Alvarez et al. 2002), to the envelope glycoproteins from *HIV-1, Hepatitis C virus* and *cytomegalovirus* (Alvarez et al. 2002; Bashirova et al. 2001; Bovin et al. 2003; Gardner et al. 2003; Halary et al. 2002), similar to DC-SIGN. L-SIGN is a liver-specific capture receptor for hepatitis C virus (Bovin et al. 2003).

Both DC-SIGN and DC-SIGNR bind ligands bearing mannose and related sugars through CRDs. The CRDs of DC-SIGN and DC-SIGNR bind $Man_9GlcNAc_2$ oligosaccharide 130- and 17-fold more tightly than mannose (Mitchell et al. 2001). Both DC-SIGN and DC-SIGNR possess a neck region, made up of multiple repeats, which supports the ligand-binding domain. Cross-linking analysis of full-length receptors expressed in fibroblasts confirms the tetrameric state of the intact receptors. The extra-cellular domain of DC-SIGN and DC-SIGNR, each comprises seven 23-residue tandem repeats, encoded by a single exon to form a coiled coil neck region. There is very high sequence identity between the repeat units, within each protein, and between DC-SIGN and DC-SIGNR. By analogy to other lectin receptors, such as the asialoglycoprotein receptors and CD23 (Bates et al. 1999; Beavil et al. 1995), it was suggested that this domain could mediate oligomerization, forming an α-helical coiled coil.

A subset of B cells in the blood and tonsils of normal donors also express DC-SIGN, which increased after stimulation in vitro with IL-4 and CD40 ligand, with enhanced expression of activation and co-stimulatory molecules CD23, CD58, CD80, and CD86, and CD22. The activated B cells captured and internalized X4 and R5 tropic strains of HIV-1, and mediated trans- infection of T cells. DC-SIGN serves as a portal on B cells for HIV-1 infection of T cells in trans. Transmission of HIV-1 from B cells to T cells through DC-SIGN pathway could be important in the pathogenesis of HIV-1 infection (Gupta and Rinaldo 2006).

36.5.2 Domain Organization of DC-SIGN and DC-SIGNR

DC-SIGN and DC-SIGNR consist of an N-terminal cytoplasmic domain, a repeat region consisting of seven 23-amino-acid tandem repeats, and a C-terminal C-type CRD that binds mannose-enriched carbohydrate modifications of host and pathogen proteins. They bind with highest affinity to larger glycans that contain 8 or 9 mannose residues (Mitchell et al. 2001; Guo et al. 2004). In addition, DC-SIGN, but not DC-SIGNR, binds to fucose-containing glycans, such as those present on the surfaces of nematode parasites (Appelmelk et al. 2003; Guo et al. 2004). The sugar-binding activity of each protein is conferred by a Ca^{2+}-dependent CRD that is located at the C terminus of the receptor polypeptide. This domain is separated from the membrane anchor by a neck region consisting of multiple 23-amino acid repeats. The neck forms an extended structure that associates to create a tetramer at the cell surface (Feinberg et al. 2001) (Fig. 36.3). Signals in the N-terminal cytoplasmic domain of DC-SIGN direct internalization of the receptor, which can thus mediate endocytosis and degradation of glycoproteins (Guo et al. 2004). DC-SIGNR lacks such signals and seems not to be a recycling receptor. Although these lectins on primary sinusoidal cells support HCV E2 binding, they are unable to support HCV entry. Lai et al. (2006) provided evidence for binding of circulating HCV with DC-SIGN and DC-SIGNR on sinusoidal endothelium within the liver allowing subsequent transfer of the virus to underlying hepatocytes in a manner analogous to DC-SIGN presentation of HIV on DCs (Lai et al. 2006).

36.5.3 Differences Between DC-SIGN and DC-SIGNR/L-SIGN

DC-SIGN and L-SIGN/DC-SIGNR genes have long been considered to share similar overall structure and ligand-binding characteristics. However, biochemical and structural studies show that they have distinct ligand-binding properties and different physiological functions. Of importance in both these genes is the presence of an extra-cellular domain consisting of an extended neck region encoded by tandem repeats that support the CRD, which plays a crucial role in influencing the pathogen-binding properties of these receptors. The notable difference between these two genes is in the extra-cellular domain. Whilst the tandem-neck-repeat region remains relatively constant in size in DC-SIGN, there is considerable polymorphism in L-SIGN. Homo-oligomerization of the neck region of L-SIGN has been shown to be important for high-affinity ligand binding, and heterozygous expression of the polymorphic variants of L-SIGN in which neck lengths differ could thus affect ligand-binding affinity. Despite DC-SIGN and DC-SIGNR bind HIV and enhance infection, comparison of these receptors reveals that they have very different physiological functions.

Screening an extensive glycan array demonstrated that DC-SIGN and DC-SIGNR have distinct ligand-binding properties. Structural and mutagenesis studies explain how both receptors bind high-mannose oligosaccharides on enveloped viruses and why only DC-SIGN binds blood group antigens, including those present on microorganisms. DC-SIGN mediates endocytosis, trafficking as a recycling receptor and releasing ligand at endosomal pH, whereas

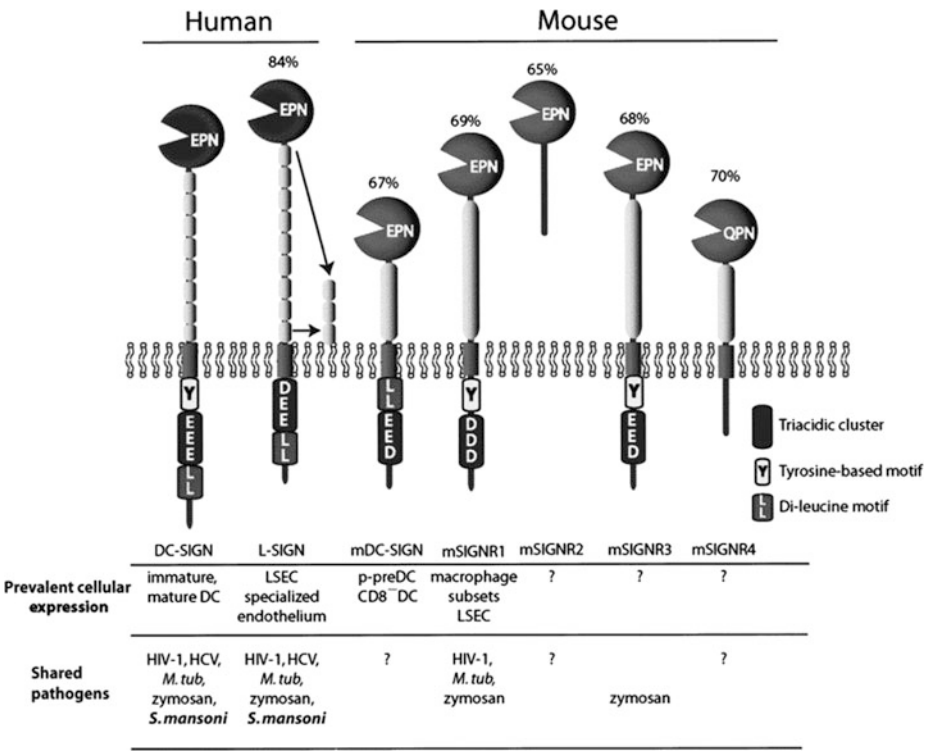

Fig. 36.3 Schematic representation of the structure, expression and binding specificities of DC-SIGN and its human and murine homologs. All SIGN homologs are transmembrane receptors, except for mSIGNR2, which is a soluble receptor. The percentage amino-acid-homology of CRDs of different homologs compared to DC-SIGN is depicted above the CRDs. Within the CRDs the highly conserved EPN sequence is essential for recognizing mannose-containing structures. All SIGN homologs contain the EPN motif, except for mSIGNR4, which has a QPN motif, indicating that mSIGNR4 may have another ligand specificity compared to the other SIGN molecules. In contrast to DC-SIGN that contains seven complete and one incomplete repeats, the number of repeats of L-SIGN is variable and varies between three and nine which is indicated with arrows. Within the cytoplasmic tail several internalization motifs are found. The di-leucin (LL) motif is thought to be important for internalization of DC-SIGN. The tyrosine-based motif and the tri-acidic cluster are also involved in internalization. However, the internalization capacities of the homologs of DC-SIGN have not been extensively explored. Based on its expression pattern, mSIGNR1 seems more similar to the homolog of L-SIGN rather than DC-SIGN whereas the expression of mDC-SIGN seems more homologous to DC-SIGN, although they are expressed by different DC subtypes. Strikingly, DC-SIGN, L-SIGN and mSIGNR1 share the ability to bind a number of pathogens (Reprinted with permission from Koppel et al. 2005 © John Wiley and Sons)

DC-SIGNR does not release ligand at low pH or mediate endocytosis. Thus, whereas DC-SIGN has dual ligand-binding properties and functions both in adhesion and in endocytosis of pathogens, DC-SIGNR binds a restricted set of ligands and has only the properties of an adhesion receptor (Guo et al. 2004). Functional studies on the effect of tandem-neck-repeat region on pathogen-binding, as well as genetic association studies for various infectious diseases and among different populations, have been reported. Worldwide demographic data of the tandem-neck-repeat region showing distinct differences in the neck-region allele and genotype distribution among different ethnic groups have been presented. These findings support the neck region as an excellent candidate acting as a functional target for selective pressures exerted by pathogens (Khoo et al. 2008). Chung et al (2010) identified Trp-258 in the DC-SIGN CRD to be essential for HIV-1 transmission. Although introduction of a K270W mutation at the same position in L-SIGN was insufficient for HIV-1 binding, an L-SIGN mutant molecule with K270W and a C-terminal DC-SIGN CRD subdomain transmitted HIV-1 (Chung et al. 2010).

36.5.4 Recognition of Oligosaccharides by DC-SIGN and DC-SIGNR

Both DC-SIGN and DC-SIGNR bind mannose bearing ligands and related sugars through CRDs. The CRDs of DC-SIGN and DC-SIGNR bind $Man_9GlcNAc_2$ oligosaccharide 130- and 17-fold more tightly than mannose. Results indicate that 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 HIV-1, which contributes to virus infection (Mitchell et al. 2001). Crystal structures of carbohydrate-recognition domains of DC-SIGN and of DC-SIGNR bound to oligosaccharide, in combination with binding studies, revealed that these receptors selectively recognize endogenous high-mannose oligosaccharides and may represent a new avenue for developing HIV prophylactics (Feinberg et al. 2001) (Fig. 36.4).

Similar to DC-SIGN, DC-SIGNR/L-SIGN can recognize high-mannose type *N*-glycans and the fucosylated glycan epitopes Lewis^{A} (Le^{a}, Galß1-3(Fuc1-4)GlcNAc-), Lewis ^{B} (Le^{b}, Fuc1-2Galß1-3(Fuc1-4)GlcNAc-) and Lewis Y (Le^{Y}, Fuc1-2Galß1-4(Fuc1-3)GlcNAc-) (Geijtenbeek et al. 2003; Guo et al. 2004; van Liempt et al. 2004). L-SIGN, however, does not bind to the Le^{X} epitope, which is one of the major ligands of DC-SIGN, although the formation of crystals between L-SIGN and Le^{X} indicates that a weak interaction is possible (Guo et al. 2004). The inability of L-SIGN to bind to Le^{X} epitopes is mainly due to the presence of a single amino acid in the CRD of L-SIGN, Ser^{363} that prevents interaction with the Fuc(1-3)GlcNAc unit in Le^{X}, but supports binding of the Fuc1-4GlcNAc moiety present in Le^{A} and Le^{B} antigens. The equivalent amino acid residue Val^{351} in DC-SIGN creates a hydrophobic pocket that strongly interacts with the Fuc(1-3/4)GlcNAc moiety of Le^{X}, other Lewis antigens, and probably LDN-F (Guo et al. 2004; van Liempt et al. 2004; van Liempt et al. 2006). The interaction of L-SIGN with *S. mansoni* egg glycoproteins and its location on liver endothelial cells suggest that L-SIGN may function in the recognition of glycan antigens of eggs that are trapped in the liver, thus contributing to glycan-specific immune responses and/or the immunopathology of schistosomiasis.

36.5.5 Extended Neck Regions of DC-SIGN and DC-SIGNR

Two different crystal forms of truncated DC-SIGNR comprising two neck repeats and the CRD reveal that the CRDs are flexibly linked to the neck, which contains α-helical segments interspersed with non-helical regions. Differential scanning calorimetry measurements indicated that the neck and CRDs are independently folded domains. Based on the crystal structures and hydrodynamic data, models for the full extracellular domains of the receptors have been generated. The observed flexibility of the CRDs in the tetramer, combined with reported data on the specificity of these receptors, suggests an important role for oligomerization in the recognition of endogenous glycans, in particular those present on the surfaces of enveloped viruses recognized by these proteins (Feinberg et al. 2005, 2009). To understand the tetramer-based ligand binding avidity, Snyder et al. (2005)

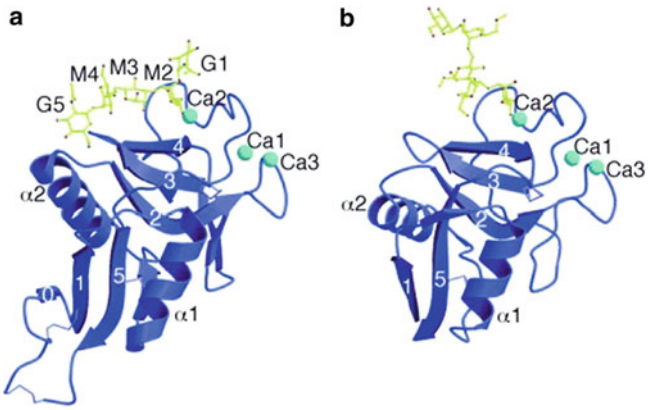

Fig. 36.4 Structure of CRD of DC-SIGN bound to $GlcNAc_2Man_3$. (**a**) Ribbon diagram of the DC-SIGN CRD (*blue*), with the bound oligosaccharide shown in a *ball-and-stick* representation (*yellow-green*, bonds and carbon atoms; *red*, oxygen; *blue*, nitrogen). Oligosaccharide residues are shown with the single letter code G for GlcNAc and M for mannose. Large cyan spheres are three Ca^{2+} ions. Disulfide bonds are shown in *pink*. The DC-SIGNR complex is very similar, except that the fourth disulfide connecting the NH_2 and COOH termini is not visible in either copy. (**b**) Rat serum mannose-binding protein bound to a high-mannose oligosaccharide. The color scheme is same as in (**a**) (Adapted from Feinberg et al. 2001 © American Association for the Advancement of Science)

determined the crystal structure of DC-SIGNR with its last repeat region and showed that compared to the carbohydrate-bound CRD structure, there are conformational differences in the calcium and carbohydrate coordination loops of CRD, an additional disulfide bond between the N and the C termini of the CRD, and a helical conformation for the last repeat. Snyder et al. (2005) generated a tetramer model for DC-SIGN/R using homology modeling and proposed a ligand-recognition index to identify potential receptor ligands. Polymorphisms associated with the length of the extracellular neck region of DC-SIGNR have been linked to differences in susceptibility to infection by enveloped viruses. The heterotetramers provide a molecular basis for interpreting the way polymorphisms affect interactions with viruses (Guo et al. 2006).

CRDs in DC-SIGN and DC-SIGNR are projected from the membrane surface by extended neck domains containing multiple repeats of a largely conserved 23-amino-acid sequence motif. The repeats are largely α-helical. Based on the structure and arrangement of the repeats in the crystal, the neck region can be described as a series of four-helix bundles connected by short, non-helical linkers. Combining the structure of the isolated neck domain with a overlapping structure of the distal end of the neck region with the CRDs attached provides a model of the almost-complete extracellular portion of the receptor. The organization of the neck suggests how CRDs may be disposed differently in DC-SIGN compared with DC-SIGNR and in variant forms of DC-SIGNR assembled from polypeptides with different

numbers of repeats in the neck domain (Feinberg et al, 2009).

CRD of Langerin with Structural Similarity with DC-SIGN: Though DCs are thought to mediate HIV-1 transmission, yet it is becoming evident that different DC subsets at the sites of infection have distinct roles. In the genital tissues, two different DC subsets are present: the LCs and the DC-SIGN$^+$-DCs. Although DC-SIGN$^+$-DCs mediate HIV-1 transmission, recent data demonstrate that LCs prevent HIV-1 transmission by clearing invading HIV-1 particles. However, this protective function of LCs is dependent on the function of Langerin: blocking Langerin function by high virus concentrations enables HIV-1 transmission by LCs. A better understanding of the mechanism of these processes is crucial to understand and develop strategies to combat transmission (de Witte et al. 2008b). The CRD of human Langerin was examined by X-ray analyses for apo-Langerin and for complexes with mannose and maltose. The fold of the Langerin CRD resembles that of DC-SIGN. However, especially in the long loop region (LLR), which is responsible for carbohydrate-binding, two additional secondary structure elements are present: a 3(10) helix and a small β-sheet arising from the extended β-strand 2, which enters into a hairpin and a new strand β2'. However, the crystal structures in presence of maltose and mannose revealed two sugar-binding sites. One is calcium-dependent and structurally conserved in the C-type lectin family whereas the second one represents a calcium-independent type. Based on these data, the differences in binding behavior between Langerin and DC-SIGN with respect to the Lewis X carbohydrate antigen and its derivatives has been explained (Chatwell et al. 2008).

36.5.6 Signaling by DC-SIGN through Raf-1

Adaptive immune responses by DCs are critically controlled by Toll-like receptor (TLR) function. Little is known about modulation of TLR-specific signaling by other pathogen receptors. DC-SIGN has gained an exponential increase in attention because of its involvement in multiple aspects of immune function. Besides being an adhesion molecule, particularly in binding ICAM-2 and ICAM-3, it is also crucial in recognizing several endogenous and exogenous antigens. Additionally, the intracellular domain of DC-SIGN includes molecular motifs, which enable the activation of signal transduction pathways involving serine and threonine kinase Raf-1 and subsequent modulation of DC-maturation status, through direct modification of nuclear factor Nf-kB in DCs. DC-SIGN modulates TLR signaling at the level of the transcription factor NF-kB.

The DC-SIGN has emerged as a key player in the induction of immune responses against numerous pathogens by modulating TLR-induced activation. Upon DC-SIGN engagement by mannose- or fucose-containing oligosaccharides, the latter leads to a tailored Toll-like receptor signaling, resulting in an altered DC-cytokine profile and skewing of Th1/Th2 responses. Gringhuis et al. (2007) demonstrated that pathogens trigger DC-SIGN on human DCs to activate Raf-1, which subsequently leads to acetylation of the NF-kB subunit p65, but only after TLR-induced activation of NF-kB. Acetylation of p65 both prolonged and increased IL10 transcription to enhance anti-inflammatory cytokine responses. Different pathogens such as *M. tuberculosis, M. leprae, Candida albicans, measles virus,* and *HIV-1* interacted with DC-SIGN to activate the Raf-1-acetylation-dependent signaling pathway to modulate signaling by different TLRs. Thus, this pathway is involved in regulation of adaptive immunity by DCs to bacterial, fungal, and viral pathogens (Gringhuis et al. 2007). In addition, other DC-SIGN-ligands induce different signaling pathways downstream of Raf-1, indicating that DC-SIGN-signaling is tailored to the pathogen.

36.6 Functions of DC-SIGN

36.6.1 DC-SIGN Supports Immune Response

Immature DCs are recruited from blood into tissues to patrol for foreign antigens. Cells expressing DC-SIGN stable transfectants were able to mediate phagocytosis of *E. coli*. Ca^{2+} binding sites in the CRD of DC-SIGN were involved in phagocytosis of bacteria as well as multimerization of DC-SIGN, and neck region played a role in efficiency of binding to microbes as well as multimerization of the protein (Iyori et al. 2008; Valera et al. 2008). While analyzing early stages of DC-SIGN-mediated endocytosis, Cambi et al. (2009) demonstrated that both membrane cholesterol and dynamin are required and that DC-SIGN-mediated internalization occurs via clathrin-coated pits. Electron microscopy studies confirmed the involvement of DC-SIGN in clathrin-dependent HIV-1 internalization by DCs. Recent studies showed that some functions of decidual dendritic cells appear to be essential for pregnancy. In humans, decidual dendritic cells are identifiable by their expression of DC-SIGN. In normal decidua, DC-SIGN$^+$ cells expressed antigens associated with immature myeloid dendritic cells. In samples from spontaneous abortions, the decidual DC-SIGN$^+$ cells at a significantly lower proportion compared to normal pregnancies seem to play a role in pathological pregnancy outcomes (Tirado-González et al. 2010).

The dendritic cell-specific DC-SIGN internalizes antigen for presentation to T cells. After antigen uptake and

processing, DCs mature and migrate to the secondary lymphoid organs where they initiate immune responses. As an adhesion molecule, DC-SIGN is able to mediate rolling and adhesion over endothelial cells under shear flow. The normal functions of DC-SIGN and DC-SIGNR include binding to ICAM-2 and ICAM-3. Binding of DC-SIGN to ICAM-2 on endothelial cells facilitates chemokine-induced DC extravasation; binding to ICAM-3 on T lymphocytes provides the initial step for establishing cell-mediated immunity (Liu and Zhu 2005). DCs could activate arrest T cells in the lymph node, but the mechanism is poorly understood. Studies showed that binding of DC-SIGN to both carcinoembryonic antigen (CEA)-related cell adhesion molecule 1 (CEACAM1) and Mac1 was required to establish cellular interaction between DCs and neutrophils, and such interaction promoted T cell proliferation and transformation to Th1 cells (van Gisbergen et al. 2005a, c). Subpopulations of human macrophages express DC-SIGN, to which Le^x-carrying CEACAMs may modulate the immune response in normal tissues such as the human placenta or in malignant tumors, for example in colorectal, pancreatic or lung carcinomas (Samsen et al. 2010). Reports suggest that DCs participate in the contact between itself and resting T cells, and also in T cell activation, and such effect is related to its cytoplasmic ITAM signal transduction (Engering et al. 2002). Martinez et al. discovered that DC-SIGN could promote CD3-activated T cells to produce IL-2 and receive a strong TCR signal, thus strengthening TCR-APC interaction and enhancing immune response (Martinez et al. 2005). Inhibiting DC-SIGN on DCs could reduce T cell proliferation and inhibit co-stimulator CD11c, CD83, CD80 and CD86 expression. Such effects are achieved by NF-κB signaling pathway (Zhou et al. 2006a). Recent evidences suggest that there is a cross-talk between DC-SIGN together with its CLRs and TLRs, and such crosstalk could lead to immune activation or T cells depression (Geijtenbeek et al. 2004; Gantner et al. 2003; Zhou et al. 2006b).

36.6.2 DC-SIGN Recognizes Pathogens

Viral and Bacterial Antigens DC-SIGN plays an important role in recognizing and capturing pathogens, DC migration and initiation of T cell responses. The role of DC-SIGN as a broad pathogen receptor has been well established (Geijtenbeek et al. 2000a; Alvarez et al. 2002; Colmenares et al. 2002; Cambi et al. 2003; Geijtenbeek et al. 2003; Lozach et al. 2003). In addition, DC-SIGN functions as a cell adhesion receptor mediating the interaction between DCs and resting T cells by binding to ICAM-3, and the transendothelial migration of DCs by binding to ICAM-2 (Geijtenbeek et al. 2000b). DC-SIGN has been identified as a receptor for HIV-1, HCV, Ebola virus, CMV, dengue virus, and the SARS coronavirus. Evidences suggest that DC-SIGN can function both as an adhesion receptor and as a phagocytic pathogen-recognition receptor, similar to the Toll-like receptors. Although major differences in the cytoplasmic domains of these receptors might predict their function, findings show that differences in glycosylation of ligands can dramatically alter C-type lectin-like receptor usage (Cambi and Figdor 2003; Engering et al. 2002). As a pathogen receptor, DC-SIGN displays affinity for high mannose moieties and functions as an internalization receptor for *HIV-1*, *hepatitis C virus, Mycobacterium tuberculosis, Helicobacter pylori,* and *Schistosoma manson, Leishmania*, and *Candida albicans* (Appelmelk et al. 2003; Bashirova et al. 2003a; Feinberg et al. 2001; Jack et al. 2001; Colmenares et al. 2004; Geijtenbeek et al. 2000a; Lozach et al. 2003; van Kooyk and Geijtenbeek 2003) and other pathogens that express mannose-containing carbohydrates (Alvarez et al. 2002; Halary et al. 2002; Tassaneetrithep et al. 2003; Zhou et al. 2008). The DC-SIGN molecule is used by HIV to attach to DCs in the genitourinary tract and rectum. Geitjenbeek et al. (2000a, c) suggest that DCs then carry HIV particles to lymph nodes, where the infection of T lymphocytes via receptors such as CD4 and CCR5 may occur. The virus may remain bound to DC-SIGN for protracted periods. DC-SIGN may deliver bound HIV to permissive cell types, mediating infection with high efficiency.

In vitro, DC-SIGN specifically interacts with *S. pneumoniae* serotype 3 and 14 in contrast to other serotypes such as 19F. While DC-SIGN interacts with *S. pneumoniae* serotype 14 through a ligand expressed by the capsular polysaccharide, the binding to *S. pneumoniae* serotype 3 appeared to depend on an as yet unidentified ligand (Koppel et al. 2005b). Leptospirosis is a global zoonotic disease, caused by pathogenic Leptospira species including *Leptospira interrogans* that causes public health and livestock problems. *L. interrogans* binds DC-SIGN and induces DCs maturation and cytokine production, which should provide new insights into cellular immune processes during leptospirosis (Gaudart et al. 2008).

Schistosoma Mansoni Antigens: Schistosomiasis is a human parasitic disease caused by helminths of the genus *Schistosoma*. *Schistosoma mansoni* synthesizes a multitude of carbohydrate complexes, which include both parasite-specific glycan antigens, as well as glycan antigens that are shared with the host. One example for a host-like glycan is the Le^X epitope Galß1-4(Fuc1-3)GlcNAc, which is expressed in all schistosomal life stages, but also on human leukocytes. Glycan antigens expressed by schistosomes induce strong humoral and cellular immune responses in their host (Meyer et al. 2007). The recognition of

carbohydrates is mediated by DC-SIGN, and DC-SIGNR bind to glycans of *S. mansoni* soluble egg antigens (SEA) (van Die et al. 2003; van Liempt et al. 2004; Meyer et al. 2005; Saunders et al. 2009; van Die and Cummings 2006, 2010). L-SIGN binds both SEA and egg glycosphingolipids, and can mediate internalization of SEA by L-SIGN expressing cells. L-SIGN predominantly interacts with oligomannosidic *N*-glycans of SEA. L-SIGN binds to a glycosphingolipid fraction containing fucosylated species with compositions of $Hex_1HexNAc_{5-7}dHex_{3-6}Cer$. Results indicate that L-SIGN recognizes both oligomannosidic *N*-glycans and multiply fucosylated carbohydrate motifs within *Schistosoma* egg antigens, which demonstrate that L-SIGN has a broad but specific glycan recognition profile (Meyer et al. 2007).

DC-SIGN Captures Also Fungi: The DC-SIGN is located in the submucosa of tissues, where they mediate HIV-1 entry. Interestingly, the pathogen *C. albicans*, the major cause of hospital-acquired fungal infections, penetrates at similar submucosal sites. The DC-SIGN is able to bind *C. albicans* both in DC-SIGN-transfected cell lines and in human monocyte-derived DC. Moreover, in immature DC, DC-SIGN was able to internalize *C. albicans* in specific DC-SIGN-enriched vesicles, distinct from those containing the ManR. These results demonstrated that DC-SIGN is an exquisite pathogen-uptake receptor that captures not only viruses but also fungi (Cambi et al. 2003). However, DC-SIGN regulation in monocyte-derived macrophages does not singly predict the transmission potential of this cell type (Bashirova et al. 2003; Chehimi et al. 2003).

Microbial Uptake Capacities of DC-SIGN, SIGNR1, SIGNR3 and Langerin: Using transfected non-macrophage cell lines, Takahara et al. (2004) compared the polysaccharide and microbial uptake capacities of three lectins—DC-SIGN, SIGNR1 and SIGNR3—to another homolog mLangerin. Each molecule shares a potential mannose-recognition EPN-motif in its carbohydrate recognition domain. Using an anti-Tag antibody, it was found that each molecule could be internalized, although the rates differed. However, mDC-SIGN was unable to take up FITC-dextran, FITC-ovalbumin, zymosan or heat-killed *C. albicans*. The other three lectins showed distinct carbohydrate recognition properties. Furthermore, only SIGNR1 was efficient in mediating the capture by transfected cells of Gram-negative bacteria, such as *E. coli and S. typhimurium,* while none of the lectins tested were competent to capture Gram-positive bacteria, *S. aureus*. Therefore, these homologous C-type lectins have distinct recognition patters for microbes despite similarities in the carbohydrate recognition domains (Takahara et al. 2004).

36.6.3 DC-SIGN as Receptor for Viruses

DC-SIGN and L-SIGN can function as attachment receptors for *Sindbis (SB) virus*, an arbovirus of the Alphavirus genus. DC-SIGN is a universal pathogen receptor and can be used by *hepatitis C virus* (HCV) and other viral pathogens including *Ebola* virus, *cytomegalovirus* (CMV), *and Dengue virus* to facilitate infection by a mechanism that is distinct from that of HIV-1, leading to inhibition of the immunostimulatory function of DC and, hence, promotion of pathogen survival. Reports show that DC-SIGN not only plays a role in entry into DC, but HCV E2 interaction with DC-SIGN might also be detrimental to the interaction of DC with T cells during antigen presentation (Zhou et al. 2008). The surface membrane glycoprotein of *Borna disease virus* (BDV) is a polypeptide of 57 kDa and N-glycosylated to a precursor glycoprotein (GP) of about 94 kDa. Analysis showed that the precursor GP contains only mannose-rich N-glycans (Kiermayer et al. 2002) and has the potential to bind with DC-SIGN, L-SIGN in addition to other mannose spectic lectins discussed in different Chapters.

Reduced expression of DC-SIGN in spleen specifically characterizes pathogenic forms of simian immunodeficiency virus (SIV) infection, correlates with disease progression, and may contribute to SIV pathogenesis (Yearley et al. 2008).

N-glycan Status Modifies Virus Interaction: The virus-producing cell type is an important factor in dictating both N-glycan status and virus interaction with DC-SIGN/DC-SIGNR (Lin et al. 2003). In contrast, viruses bearing Ebola (Zaire strain) and Marburg (Musoke strain) envelope glycoproteins bind at significantly higher levels to immobilized MBL compared with virus particles pseudo typed with vesicular stomatitis virus glycoprotein or with no virus glycoprotein. Importantly, the tetrameric complexes, in contrast to DC-SIGN monomers, bind with high affinity to high mannose glycoproteins such as mannan or HIV gp120 suggesting that such an assembly is required for high affinity binding of glycoproteins to DC-SIGN (Bernhard et al. 2004).

36.6.4 HIV-1 gp120 and Other Viral Envelope Glycoproteins

DC-SIGN has been described as an attachment molecule for human HIV-1 with the potential to mediate its transmission. About half of the carbohydrates on gp120 are terminally mannosylated, a pattern common to many pathogens. The DC-SIGN binds to HIV and SIV gp120 and mediates the binding and transfer of HIV from monocyte-derived dendritic cells (MDDCs) to permissive

T cells (Geijtenbeek et al. 2002c). However, DC-SIGN binding to HIV gp120 may also be carbohydrate independent. Hong et al. (2002) formally demonstrated that gp120 binding to DC-SIGN and MDDCs is largely if not wholly carbohydrate dependent.

DC-SIGN was mainly expressed in tubular epithelial cells and DC-SIGN$^+$ DCs were primarily distributed in renal tubulo-interstitial areas during the early stage of nephritis. In a rat model of chronic renal interstitial fibrosis, there was a significant correlation of DC-SIGN expression with DC-SIGN$^+$ DC distribution and the degree of tubulo-interstitial lesion. DC-SIGN plays an important role in DC-mediated renal tubular interstitial lesions induced by immuno-inflammatory responses (Zhou et al. 2009). HIV-1 infection of renal cells has been proposed to play a role in HIV-1-associated nephropathy. The HIV-1 internalization was DC-SIGN receptor mediated. It appeared that HIV-1 routing occurred through nonacid vesicular compartments and the clathrin-coated vesicles and caveosomes may not be contributing to HIV-1-associated membrane traffic (Mikulak et al. 2010). However, Hatsukari et al. (2007) showed no expression of DC-SIGN, or mannose receptors in tubular cells and suggested that DEC-205 (Chap.15 and Chap. 35) acts as an HIV-1 receptor that mediates internalization of the virus into renal tubular cells (HK2), from which the virus can be rescued and disseminated by encountering immune cells.

Herpes Simplex Virus and Human Herpes Virus 8: Of the two Herpes simplex virus (HSV) subtypes described, HSV-1 causes mainly oral-facial lesions, whilst HSV-2 is associated with genital herpes. HSV-1 and -2 both interact with DC-SIGN. Analyses demonstrated that DC-SIGN interacts with the HSV glycoproteins gB and gC. In another setting, human herpesvirus 8 (HHV-8) is the etiological agent of Kaposi's sarcoma, primary effusion lymphoma, and some forms of multicentric Castleman's disease. DC-SIGN is an entry receptor for HHV-8 on DC and macrophages. The infection of B cells with HHV-8 resulted in increased expression of DC-SIGN and a decrease in the expression of CD20 and MHC-I. It was indicated that the expression of DC-SIGN is essential for productive HHV-8 infection of and replication in B cells (de Jong et al. 2008; Rappocciolo et al. 2006, 2008).

West Nile Virus: West Nile virus (WNV), a mosquito-borne flavivirus, has recently emerged in North America. The elderly are particularly susceptible to severe neurological disease and death from infection with this virus. DC-SIGN enhances infection of cells by West Nile virus (WNV) glycosylated strains, which may at least in part explain the higher pathogenicity of glycosylated L1 strains versus most non-glycosylated L2 strains (Martina et al. 2008). Kong et al. (2008) found that the binding of the glycosylated WNV envelope protein to DC-SIGN leads to a reduction in the expression of TLR3 in macrophages from young donors via signal transducer and activator of transcription 1 (STAT1)-mediated pathway. This signaling is impaired in the elderly, and the elevated levels of TLR3 result in an elevation of cytokine levels. This alteration of the innate immune response with aging may contribute to the permeability of blood–brain barrier and a possible mechanism for the increased severity of WNV infection in aged individuals.

Corona Virus Infection and SARS: The MBL deficiency is a susceptibility factor for acquisition of severe acute respiratory syndrome (SARS) corona virus (CoV) (Ip et al. 2005). On similar pattern, entry of the serotype II feline corona virus strains feline infectious peritonitis virus (FIPV) and DF2 into nonpermissive mouse 3T3 cells could be rescued by the expression of human DC-SIGN and the infection of a permissive feline cell line (Crandall-Reese feline kidney) was markedly enhanced by the overexpression of DC-SIGN. Treatment with mannan considerably reduced infection of feline monocyte-derived cells expressing DC-SIGN, indicating a role for FIPV infection in vivo (Regan and Whittaker 2008).

Dengue Virus Envelope Glycoprotein and DC-SIGN: Dengue virus (DV) primarily targets immature DCs after a bite by an infected mosquito vector. Navarro-Sanchez et al. (2003) showed that DC-SIGN is a binding receptor for DV that recognizes N-glycosylation sites on the viral E-glycoprotein and allows viral replication. Mosquito-cell-derived DVs may have differential infectivity for DC-SIGN-expressing cells. There is evidence that infection of immature myeloid DCs plays a crucial role in dengue pathogenesis and that the interaction of the viral envelope E glycoprotein with DC-SIGN is a key element for their productive infection (Kwan et al. 2008).

Measles Virus Targets DC-SIGN: DCs are involved in the pathogenesis of measles virus (MV) infection by inducing immune suppression and possibly spreading the virus from the respiratory tract to lymphatic tissues. The DC-SIGN is the receptor for laboratory-adapted and wild-type MV strains. The ligands for DC-SIGN are both MV glycoproteins F and H. DC-SIGN was found important for the infection of immature DCs with MV, since both attachment and infection of immature DCs with MV were blocked in the presence of DC-SIGN inhibitors. Moreover, MV might not only target DC-SIGN to infect DCs but may also use DC-SIGN for viral transmission and immune suppression (de Witte et al. 2006). Thus, DCs play a prominent role during the initiation, dissemination, and clearance of MV infection (de Witte et al. 2008a).

36.7 Subversion and Immune Escape Activities of DC-SIGN

36.7.1 The entry and dissemination of viruses can be mediated by DC-SIGN

Dendritic cells are likely the first cells to encounter invading pathogens. The entry and dissemination of viruses in several families can be mediated by DC-SIGN. Terminal mannoses at positions 2 or 3 in the trisaccharides are the most important moiety and present the strongest contact with the binding site of DC-SIGN (Reina et al. 2008). Although, the mechanism of DC-SIGN and HIV-1 interaction remains unclear, Smith et al. (2007) identified a cellular protein that binds specifically to the cytoplasmic region of DC-SIGN and directs internalized virus to the proteasome degradation. This cellular protein, leukocyte-specific protein 1 (LSP1) is an F-actin binding protein involved in leukocyte motility and found on the cytoplasmic surface of the plasma membrane. LSP1 interacted specifically with DC-SIGN and other C-type lectins, but not the inactive mutant DC-SIGNΔ35, which lacks a cytoplasmic domain and shows altered virus transport in DCs. Thus, LSP1 protein facilitates virus transport into the proteasome after its interaction with DC-SIGN through its interaction with cytoskeletal proteins. Thus, it has been proposed that attachment of HIV to DC-SIGN enables the virus to hijack cellular transport processes to ensure its transmission to adjacent T cells.

Interestingly, not all interactions between DC-SIGN receptors and pathogenic ligands have beneficial results. During the interaction between body and pathogens or tumors, the latter could escape immune surveillance and survive. Such mechanism is related to suppressions of DCs by DC-SIGN. Thus, though DCs are vital in the defense against pathogens, it is becoming clear that some pathogens subvert DC functions to escape immune surveillance. It appears that some pathogens have evolved immunoevasive or immunosuppressive activities through receptors such as DC-SIGN. For example, HIV-1 targets the DC-SIGN to hijack DCs for viral dissemination. Binding to DC-SIGN protects HIV-1 from antigen processing and facilitates its transport to lymphoid tissues, where DC-SIGN promotes HIV-1 infection of T cells. Studies have also demonstrated that different ligand binding and/or sensing receptors collaborate for full and effective immune responses (McGreal et al. 2005; van Kooyk et al. 2003; Wang et al. 2004). It was also demonstrated that DC-SIGN efficiently reduces the amount of gp120 present on the cell plasma membrane, and completely strips off gp120 from the virions produced by the host cells, suggesting that blockage of HIV budding is due to internalization of gp120 by DC-SIGN (Wang and Pang 2008; Solomon Tsegaye and Pohlmann 2010).

Perhaps, binding of DC-SIGN to gp120 may facilitate or stabilize these transitions. Further studies demonstrated that HIV-1 would lose its activity if it is kept in vitro for 24 h, but DC-SIGN-bound HIV-1 could be kept within DCs for more than 4 days, allowing incoming virus to persist for 25 days before infecting target (Zhou et al. 2004). The mechanism of DC-SIGN prolongating viral infectivity is still poorly understood (Lozach et al. 2005). Thus, HIV sequestration by and stimulation of DC-SIGN helps HIV evade immune responses and spread to cells (Hodges et al. 2007; Marzi et al. 2007).

Many Pathogens Target DC-SIGN to Escape Host Immunity: For Hepatitis C Virus, both DC-SIGN and L-SIGN are known to bind envelope glycoproteins E1 and E2. Soluble DC-SIGN and L-SIGN specifically bound HCV virus-like particles. It is also speculated that HBV exploits mannose trimming as a way to escape recognition by DC-SIGN and thereby subvert a possible immune activation response (Op den Brouw et al. 2008). Reduced expression of DC-SIGN in spleen specifically characterizes pathogenic forms of SIV infection, correlates with disease progression, and may contribute to SIV pathogenesis (Yearley et al. 2008). DC-SIGN acts as a capture or attachment molecule for avian H5N1 virus, and mediates infections in cis and in trans (Wang et al. 2008b). Mittal et al. (2009) demonstrated that *Enterobacter sakazakii* (ES) targets DC-specific DC-SIGN to survive in myeloid DCs for which outer membrane protein A expression in ES is critical, although it is not required for uptake. ES interaction with DC-SIGN seems to subvert the host immune responses by disarming MAPK pathway in DCs (Mittal et al. 2009). *Yersinia pestis* is the etiologic agent of bubonic and pneumonic plagues. It is speculated that *Y. pestis* hijacks DCs and alveolar macrophages, in order to be delivered to lymph nodes. DC-SIGN is a receptor for *Y. pestis* that promotes phagocytosis by DCs in vitro (Zhang et al. 2008b). Accumulating evidence supports that certain pathogens target DC-SIGN to escape host immunity. Unlike certain other host pathogen interactions, activation of DCs by *B. pseudomallei* is not dependent on DC-SIGN. Evidence also indicates that the LPS mutant that binds DC-SIGN has a suppressive effect on DC cytokine production (Charoensap et al. 2008).

36.7.2 DC-SIGN and Escape of Tumors

Recognition of Tumor Glycans: Dendritic cells play an important role in the induction of antitumor immune responses. Glycosylation changes during malignant transformation create tumor-specific carbohydrate structures that interact with C-type lectins on DCs. Studies suggest that tumor glycoproteins, such as carcinoembryonic antigen (CEA) and MUC-1, indeed interact with DC-SIGN and

macrophage galactose-type lectin on APCs. DC-SIGN has been detected on immature DCs that were associated with melanoma and myxofibrosarcoma (Soilleux et al. 2003; Vermi et al. 2003). The consequences for anti-cancer immunity or tolerance induction can be extrapolated from the function of C-type lectins in pathogen recognition and antigen presentation. In addition, in vivo studies in mice demonstrated the potency of targeting antigens to C-type lectins on APCs for anti-tumor vaccination strategies (Aarnoudse et al. 2006).

DC-SIGN and Escape of Tumors: DC-SIGN has been related to immune escape of tumors (van Gisbergen et al. 2005b; Gijzen et al. 2008). Immature DCs are located intratumorally within colorectal cancer and intimately interact with tumor cells, whereas mature DCs are present peripheral to the tumor. The majority of colorectal cancers over-express CEA and malignant transformation changes the glycosylation of CEA on colon epithelial cells, resulting in higher levels of Le^X and de novo expression of Le^Y on tumor-associated CEA. Since DC-SIGN has high affinity for nonsialylated Lewis antigens, it is possible that DC-SIGN is involved in recognition of colorectal cancer cells by DCs. It was shown that immature DCs within colorectal cancer express DC-SIGN, which mediates these interactions through binding of Le^X/ Le^Y carbohydrates on CEA of colorectal cancer cells. In contrast, DC-SIGN does not bind CEA expressed on normal colon epithelium due to low levels of Lewis antigens. This indicates that DCs may recognize colorectal cancer cells through binding of DC-SIGN to tumor-specific glycosylation on CEA. Similar to pathogens that target DC-SIGN to escape immunosurveillance, tumor cells may interact with DC-SIGN to suppress DC functions (van Gisbergen et al. 2005b). At the same time, tumor cells can suppress DC maturation by DC-SIGN and escape immune surveillance. Bogoevska et al. (2006) showed that CEA-related cell adhesion molecule 1 (CEACAM1) selectively attaches and specifically interacts with DC-SIGN, and participates in cancer development. DC-SIGN is involved in the interaction of DCs with colorectal tumor SW1116 cells through the recognition of aberrantly glycosylated forms of Le^a/Le^b glycans on CEA and CEACAM. DC-SIGN ligands containing Le^a/Le^b glycans are also highly expressed on primary cancer colon epithelia but not on normal colon epithelia, and DC-SIGN is suggested to be involved in the association between DCs and colorectal cancer cells in situ by DC-SIGN recognizing these cancer-related Le glycan ligands. Observations imply that colorectal carcinomas affecting DC function and differentiation through interactions between DC-SIGN and colorectal tumor-associated Le glycans may induce generalized failure of a host to mount an effective antitumor response (Nonaka et al. 2008). In acute lymphoblastic leukemia (ALL), aberrant glycosylation of blast cells can alter their interaction with DC-SIGN and L-SIGN, thereby affecting their immunological elimination. High binding of B-ALL peripheral blood cells to DC-SIGN and L-SIGN correlates with poor prognosis. Apparently, when B-ALL cells enter the blood circulation and are able to interact with DC-SIGN and L-SIGN the immune response is shifted toward tolerance (Gijzen et al. 2008).

The role of DCs in progression of primary cutaneous T-cell lymphoma (CTCL) is not established. Schlapbach et al. (2010) found a significant infiltration of CTCL lesions by immature DC-SIGN$^+$ DCs with close contact to tumor cells. Matured and activated DCs were only rarely detected in lesions of CTCL. The preponderance of immature DC-DIGN$^+$ DCs in contact with regulatory T cells in lesions of CTCL points to an important role of this subset in the host's immune reaction to the malignant T cells. Since these immature DCs are known to induce immunotolerance, they might play a role in the mediation of immune escape of the proliferating clone (Schlapbach et al. 2010). In contrast, studies showed that there are CEA specific T cells in colorectal cancer patients which have anti-tumor effects (Nagorsen et al. 2000). Such results suggest that DCs could recognize and bind to colorectal tumor cells by DC-SIGN and participate in anti-tumor immune response.

36.7.3 *Mycobacterial* Carbohydrates as Ligands of DC-SIGN, L-SIGN and SIGNR1

Mycobacteria, including *M. tuberculosis* (Mtb), are surrounded by a loosely attached capsule that is mainly composed of proteins and polysaccharides. Although the chemical composition of the capsule is relatively well studied, its biological function is only poorly understood. *M. tuberculosis*, the causative agent of tuberculosis (TB), is recognized by pattern recognition receptors on macrophages and DCs, thereby triggering phagocytosis, antigen presentation to T cells and cytokine secretion. Mtb spreads through aerosol carrying them deep into the lungs, where they are internalized by phagocytic cells, such as neutrophils (PMNs), DCs, and macrophages. The DC- Mtb manipulates cells of the innate immune system to provide the bacteria with a sustainable intracellular niche (Schaefer et al. 2008). Mannosylated moieties of the mycobacterial cell wall, such as mannose-capped lipoarabinomannan (ManLAM) or higher-order phosphatidylinositol-mannosides (PIMs) of Mtb, were shown to bind to DC-SIGN on immature DCs and macrophage subpopulations. This interaction reportedly impaired dendritic cell maturation, modulated cytokine secretion by phagocytes and dendritic cells and was postulated to cause suppression of protective immunity to TB. However, experimental Mtb infections in mice transgenic for human DC-SIGN revealed

that, instead of favoring immune evasion of mycobacteria, DC-SIGN may promote host protection by limiting tissue pathology. Furthermore, infection studies with mycobacterial strains genetically engineered to lack ManLAM or PIMs demonstrated that manLAM/PIM-DC-SIGN interaction was not critical for cytokine secretion in vitro and protective immunity in vivo (Ehlers 2010).

Reports suggest that *M. tuberculosis* targets DC-SIGN to inhibit the immuno-stimulatory function of DC through the interaction of the mycobacterial ManLAM to DC-SIGN, which prevents DC maturation and induces the formation of immuno-suppressive cytokine IL-10 that helps in the survival and persistence of *M. tuberculosis* (Fig. 36.2). The pathogen-derived carbohydrate structure on ManLAM that is recognized by DC-SIGN has been identified. The synthetic mannose-cap oligosaccharides manara, (Man)2-ara and (Man)3-ara specifically bound by DC-SIGN. The human and murine DC-SIGN homolog L-SIGN and SIGNR1, respectively, also interact with mycobacteria through ManLAM. Both homologs have the highest affinity for the (Man)3-ara structure, similar to DC-SIGN. The identification of SIGNR1 as a receptor for ManLAM enabled in vivo studies to investigate the role of DC-SIGN in *M. tuberculosis* pathogenesis (Geijtenbeek et al. 2003; Tailleux et al. 2003; Koppel et al. 2004). In addition to manLAM, Mtb α-glucan is another ligand for DC-SIGN. The recognition of α-glucans by DC-SIGN is a general feature and the interaction is mediated by internal glucosyl residues. As for manLAM, an abundant mycobacterial cell wall-associated glycolipid, binding of α-glucan to DC-SIGN stimulated the production of immunosuppressive IL-10 by LPS-activated monocyte-derived DCs. This IL-10 induction was DC-SIGN-dependent and also required acetylation of NF-kB (Geurtsen et al. 2009).

The mannose cap of LAM is a crucial factor in mycobacterial virulence. Appelmelk et al. (2008) evaluated the biological properties of capless mutants of *M. marinum* and *M. bovis* BCG, made by inactivating homologs of Rv1635c and showed that its gene product is an undecaprenyl phospho-mannose-dependent mannosyltransferase. Compared with parent strain, capless *M. marinum* induced slightly less uptake by and slightly more phagolysosome fusion in infected macrophages but this did not lead to decreased survival of the bacteria in vitro, nor in vivo in zebra fish. Appelmelk et al. (2008) contradicted the current paradigm and demonstrated that mannose-capped LAM does not dominate the M.-host interaction. Although the mannose caps of the mycobacterial surface (ManLAM) are essential for the binding to DC-SIGN, genetic removal of these caps did not diminish the interaction of whole mycobacteria with DC-SIGN and DCs. Like ManLAM, Hexamannosylated PIM (6), which contains terminal α(1—>2)-linked mannosyl residues identical to the mannose cap on ManLAM showed high affinity and represents a bonafide DC-SIGN ligand but that other, as-yet-unknown, ligands dominate in the interaction between *mycobacteria* and DCs (Driessen et al. 2009) (Fig. 36.1, 36.2).

36.7.4 Decreased Pathology of Human DC-SIGN Transgenic Mice During Mycobacterial Infection

Although, the *M. tuberculosis*, the causative agent of pulmonary tuberculosis, interacts with DC-SIGN to evade the immune system, transgenic mice after high dose aerosol infection with the strain *Mtb*-H37Rv, showed massive accumulation of DC-SIGN$^+$ cells in infected lungs, reduced tissue damage and prolonged survival. Based on these results, it was proposed that instead of favoring the immune evasion of mycobacteria, human DC-SIGN may have evolved as a pathogen receptor promoting protection by limiting tuberculosis-induced pathology (Schaefer et al. 2008). Hedlund et al. (2010) demonstrated that DCs can distinguish between normal and infected apoptotic PMNs via cellular crosstalk, where the DCs can sense the presence of danger on the Mtb-infected PMNs and modulate their response accordingly. Balboa et al. (2010) showed that early interaction of Î-irradiated *M. tuberculosis* with Mo subverts DC differentiation in vitro and suggested that *M. tuberculosis* escapes from acquired immune response in tuberculosis may be caused by an altered differentiation into DC leading to a poor Mtb-specific T-cell response. Since, *M. tuberculosis* interacts with DC-SIGN to evade the immune system, the dominant Mtb-derived ligands for DC-SIGN are presently unknown, and a major role of DC-SIGN in the immune response to Mtb infection may lie in its capacity to maintain a balanced inflammatory state during chronic TB (Ehlers 2010).

36.7.5 Genomic Polymorphism of DC-SIGN (CD209) and Consequences

36.7.5.1 CD209 Genetic Polymorphism and HIV Infectivity

DC-SIGN and DC-SIGNR have been thought to play an important role in establishing HIV infection by enhancing trans-infection of CD4$^+$ T cells in the regional lymph nodes. The variation of DC-SIGNR genotypes affects the efficacy of trans-infection by affecting the amounts of the protein expressed on cell surface and augmenting the infection. A potential association of DC-SIGN and DC-SIGNR neck domain repeat polymorphism and risk of HIV-1 infection is currently under debate. Rathore et al. (2008a) showed that polymorphism in DC-SIGN neck repeats region was rare and not associated with HIV-1 susceptibility among North Indians. But sequencing analysis of DC-SIGN gene

confirmed four novel genetic variants in intronic region flanking exon 4 coding region. A total of 13 genotypes were found in DC-SIGNR neck repeat region polymorphism. Among all the genotypes, only 5/5 homozygous showed significant reduced risk of HIV-1 infection in HIV-1-exposed seronegative individuals. A unique genotype 8/5 heterozygous was also found in HIV-1 seropositive individual, which is not reported elsewhere (Rathore et al. 2008a, b).

The association of polymorphism of homolog of DC-SIGNR gene with susceptibility to virus infection suggests that the tandem-repeat polymorphisms of the DC-SIGNR gene in the Chinese Han population exhibit unique genetic characteristics not recognized earlier in the Caucasian population. Genotype 9/5 seems to be a risk factor for HIV-1 infection in the Chinese population (Wang et al. 2008a). To understand the role of DC-SIGN neck-region length variation in HIV-1 transmission, Zhang et al. (2008a) studied 530 HIV-1-positive and 341 HIV-1-negative individuals in China. The carrier frequency of a DC-SIGN allele with <5 repeat units in the neck-region was 0.9% in HIV-1-positive and 3.8% in HIV-1-negative individuals. This observation suggests that DC-SIGN variation plays a role in HIV-1 transmission. These naturally occurring DC-SIGN neck-region variants were significantly more frequent in the Chinese population than in the US population and in a worldwide population. Several transcripts of DC-SIGN have been identified, some of which code for putative soluble proteins. However, little is known about the regulation and the functional properties of such putative sDC-SIGN variants. Based on the analysis of the cytokine/chemokine content of sDC-SIGN culture supernatants, results confirmed that sDC-SIGN, like membrane DC-SIGN counterpart, may play a pivotal role in CMV-mediated pathogenesis (Plazolles et al. 2011). Variations in genes encoding virus recognition and reactivation and patients following allogeneic stem-cell transplantation suggested that two SNP (rs735240, G > A; rs2287886, C > T) in the promoter region of DC-SIGN are significantly associated with an increased risk of development of hCMV reactivation and disease. These genetic markers influence the expression levels of DC-SIGN on immature DCs, as well as infection efficiency of immature DCs by hCMV and might help to predict the individual risk of hCMV reactivation and the disease (Mezger et al. 2008).

Variations in the number of repeats in the neck region of DC-SIGN and DC-SIGNR possibly influence host susceptibility to HIV-1 infection. Chaudhary et al. (2008a) examined the SNP of DC-SIGN and DC SIGNR in healthy HIV seronegative individuals, high risk STD patients seronegative for HIV, and HIV-1 seropositive patients from northern India. DC-SIGN polymorphism was rare and genotype 7/7 was predominant in all groups studied. DC-SIGNR was highly polymorphic and 11 genotypes were observed among the different study groups. The precise role of the polymorphic variants of DC-SIGNR needs to be elucidated in the population (Chaudhary et al. 2008a, b). GG genotype of SDF-1α 3′UTR polymorphism may be associated with susceptibility to PTB in HIV-1 infected patients in south India. Genotype frequencies of DC-SIGN polymorphisms did not differ significantly between HIV patients with or without TB. A better understanding of genetic factors that are associated with TB could help target preventive strategies to those HIV patients likely to develop tuberculosis (Alagarasu et al. 2009). Whether variants in the DC-SIGN encoding CD209 gene are associated with susceptibility to or protection against HIV-1 infection or development of TB among HIV-1 infected south Indian patients, study suggests that -336G/G genotype while associated with protection against HIV-1 infection the same genotype is also associated with susceptibility to HIV-TB among south Indians (Selvaraj et al. 2009). Olesen et al. (2007) support the report that vitamin D receptor (VDR) gene SNPs modulate the risk for TB in West Africans and suggest that variation within DC-SIGN and PTX3 also affect the disease outcome.

Reports suggest that CD209 promoter SNP-336A/G exerts an effect on CD209 expression and is associated with human susceptibility to dengue, HIV-1 and tuberculosis in humans. The CD209 -336G variant allele is also associated with significant protection against tuberculosis in individuals from sub-Saharan Africa and, cases with -336GG were significantly less prone to develop tuberculosis-induced lung cavitation. Previous in vitro work demonstrated that the promoter variant -336G allele causes down-regulation of CD209 mRNA expression. This report suggests that decreased levels of the DC-SIGN receptor may be protective against both clinical tuberculosis in general and cavitory tuberculosis disease in particular. This is consistent with evidence that *Mycobacteria* can utilize DC-SIGN binding to suppress the protective pro-inflammatory immune response (Vannberg et al. 2008).

Wichukchinda et al. (2007) genotyped two SNPs in DC-SIGN promoter (-139A/G and 336A/G), a repeat number of 69 bp in Exon 4 of DC-SIGN and DC-SIGNR, and one SNP in Exon 5 of DC-SIGNR and showed that the proportion of individuals possessing a heterozygous 7/5 and 9/5 repeat and A allele at rs2277998 of DC-SIGNR in HIV-seronegative individuals of HIV-seropositive spouses was significantly higher than HIV-seropositive individuals. These associations were observed only in females but not in males. The proportion of individuals possessing the 5A haplotype in HIV-seronegative females was significantly higher than HIV-seropositive females. These associations suggested that DC-SIGNR might affect susceptibility to HIV infection by a mechanism that is different in females and males (Wichukchinda et al. 2007; Zhu et al. 2010).

36.7.5.2 Human T-Cell Lymphotropic Virus Type 1 (HTLV) Infection

DC-SIGN plays a critical role in HTLV-1 binding, transmission, and infection, thereby providing an attractive target for the development of antiretroviral therapeutics and microbicides (Jain et al. 2009). Kashima et al. (2009) evaluated four polymorphisms located in the DC-SIGN gene promoter region (positions -336, -332 -201 and -139) in DNA samples from Brazilian ethnic groups (Caucasians, Afro-Brazilian, Asians and Amerindians) to establish the population distribution of these SNPs and correlated DC-SIGN polymorphisms and infection in samples from human T-cell lymphotropic virus type 1 (HTLV-1)-infected individuals. The -336A and -139A SNPs were quite common in Asians and that the -201T allele was not observed in Caucasians, Asians or Amerindians. No significant differences were observed between individuals with HTLV-1 disease and asymptomatic patients. However, the -336A variant was more frequent in HTLV-1-infected patients. In addition, the -139A allele was found to be associated with protection against HTLV-1 infection when the HTLV-1-infected patients as a whole were compared with the healthy-control group. Kashima et al. (2009) suggested that the -139A allele might be associated with HTLV-1 infection, although no significant association was observed among asymptomatic and HAM/TSP patients. Koizumi et al. (2007) showed that RANTES -28G was associated with delayed AIDS progression, while DC-SIGN -139C was associated with accelerated AIDS progression in HIV-1-infected Japanese hemophiliacs. While analyzing DC-SIGN and DC-SIGNR polymorphisms in Caucasian Canadian and indigenous African populations, Boily-Larouche et al. (2007) found several novel nucleotide variants within regulatory 5′- and 3′-untranslated regions of the genes that could affect their transcription and translation. Study demonstrated that Africans show greater genetic diversity at these two closely-related immune loci than observed in other major population groups.

36.7.5.3 CD209 Genetic Polymorphism and Tuberculosis Disease

DC-SIGN is a receptor capable of binding and internalizing *M. tuberculosis*. The CD209 promoter single SNP-336A/G exerts an effect on CD209 expression and is associated with human susceptibility to dengue, HIV-1 and tuberculosis in humans. In vitro studies confirmed that this SNP modulates gene promoter activity. An association study was performed in Tunisian patients comprising tuberculosis and healthy controls. Sequencing of the DC-SIGN promoter region detected four polymorphisms (-939, -871, -601, and -336), but no differences in their allelic distribution were observed between the two groups. In addition, the analysis of length variation in the DC-SIGN neck region indicated extremely low levels of polymorphisms and, again, no differences between patients and controls. Results suggested neither promoter variants nor length variation in the neck region of DC-SIGN is associated with susceptibility to tuberculosis in Tunisian patients (Ben-Ali et al. 2007).

Among Caucasians patients suffering from pulmonary tuberculosis, DC-SIGN revealed no significant differences in loci -336A/G and -871A/G with controls. Analysis of MIRU-VNTR patterns identified 50 unique profiles, among which there were genotypes of the families Beijing, T. LAM, Haarlem, "Ural" (Haarlem 4) and X. Among 90 MIRU-VNTR genotypes, 42 profiles belonged to the Beijing family. Moreover, the minimum spanning tree (MST) test revealed a number of Beijing-like strains. The genotypes of the subjects affected with Beijing and Beijing-like strains and those affected with the strains of other families (non-Beijing) were compared. A significance reduction was found in the incidence of the -336G genotype among the subjects affected with Beijing strains versus those infected with non-Beijing strains at a frequency of 0.09 and 0.24, respectively (Ogarkov et al. 2007). CD209 facilitates severe acute respiratory syndrome (SARS)-coronavirus spike protein-bearing pseudotype driven infection of permissive cells in vitro. Genetic association analysis of SNP with clinico-pathologic outcomes in 824 serologic confirmed that the -336AG/GG genotype SARS patients were associated with lower LDH levels compared with the -336AA patients. High LDH levels are known to be an independent predictor for poor clinical outcome, probably related to tissue destruction from immune hyperactivity. Hence, SARS patients with the CD209 -336 AA genotype carry a 60% chance of having a poorer prognosis. This association is in keeping with the role of CD209 in modulating immune response to viral infection. The relevance of these findings for other infectious diseases and inflammatory conditions would be worth investigating (Chan et al. 2010).

36.8 SIGNR1 (CD209b): The Murine Homologues of DC-SIGN

36.8.1 Characterization

The mouse (m) DC-SIGN family consists of several homologous type II transmembrane proteins located in close proximity on chromosome 8 and having a single carboxyl terminal carbohydrate recognition domain. Initial screening of mouse cDNA libraries led to the identification of multiple mouse homologs of DC-SIGN and DC-SIGNR, designated DC-SIGN and SIGNR1 through SIGNR4 by Park et al. (2001). More murine homologs of human SIGNs have been identified, and the biochemical and cell biological properties of all murine SIGNs have been compared (Fig. 36.3). The SIGNR1 is a C-type lectin domain of murine homolog

of DC-SIGN and functions in vivo as a pathogen recognition receptor on macrophages that captures blood-born antigens, which are rapidly internalized and targeted to lysosomes for processing (Geijtenbeek et al. 2002b). In addition to five SIGNR proteins, a pseudogene, encoding a hypothetical SIGNR6, and a further two expressed proteins, SIGNR7 and SIGNR8, have been identified. Screening of a glycan array demonstrated that only mouse SIGNR3 shares with human DC-SIGN the ability to bind both high mannose and fucose-terminated glycans in this format and to mediate endocytosis. SIGNR3 is a differentiation marker for myeloid mononuclear cells and that some DCs, especially in sLNs, are possibly replenished by Ly6C(high) monocytes (Nagaoka et al. 2010). The mouse homologs of DC-SIGN have a diverse set of ligand-binding and intracellular trafficking properties, some of which are distinct from the properties of any of the human receptors (Powlesland et al. 2006).

SIGN Related 1 (SIGNR1) or CD209b is expressed at high levels on macrophages in lymphoid tissues, especially within the marginal zone of the spleen. SIGNR1 can bind and mediate the uptake of various microbial polysaccharides, including dextrans, lipopolysaccharides and pneumococcal capsular polysaccharides. SIGNR1 mediates the clearance of encapsulated pneumococcus, complement fixation via binding C1q independent of antibody and innate resistance to pneumococcal infection. Recently, SIGNR1 has also been demonstrated to bind sialylated antibody and mediate its activity to suppress autoimmunity (Silva-Martin et al. 2009). The CRD of SIGNR1 has been cloned and overexpressed in a soluble secretory form in CHO cells. The single crystal of CRD protein of SIGNR1 belonged to the monoclinic space group C2 with unit-cell parameters $a = 146.72, b = 92.77, c = 77.06$ A, $\beta = 121.66°$, allowed the collection of a full X-ray data set to a maximum resolution of 1.87 A (Silva-Martin et al. 2009).

36.8.2 Functions

SIGNR1 receptor, involved in the uptake of capsular polysaccharides (caps-PS) by APCs, is necessary for the antibody response to pneumococcal caps-PS and phosphorylcholine (PC). Moens et al. (2007) found that SIGNR1 is not involved in the IgM antibody production to PC and caps-PS serotype 3 or 14 and the IgG immune response to PC and caps-PS serotype 14. There is no direct relation between capture and uptake of caps-PS serotype 14 by SIGNR1 and the initiation of the anti-caps-PS antibody production in mice. Resident peritoneal macrophages (PEMs) express SIGNR1 on the cell surface as a major mannose receptor. These cells also ingest oligomannose-coated liposomes (OMLs) in an oligomannose-dependent manner following intraperitoneal administration. SIGNR1 on macrophages acts as a receptor for recognition of OMLs under physiological conditions (Takagi et al. 2009). Sialylated Fc from IgG require SIGNR1 which preferentially binds to 2,6-sialylated Fc compared with similarly sialylated, biantennary glycoproteins, suggesting that a specific binding site is created by sialylation of IgG Fc. A human DC-SIGN displays a binding specificity similar to SIGNR1 but differs in its cellular distribution. These studies thus identify an antibody receptor specific for sialylated Fc, and present the initial step that is triggered by intravenous Ig to suppress inflammation (Anthony et al. 2008).

SIGNR1 also interacts with *M. tuberculosis* similar to DC-SIGN. Peritoneal macrophages from SIGNR1 deficient (KO) mice produce less IL-10 upon stimulation with ManLAM than those from wild-type mice, suggesting that the interaction of ManLAM with SIGNR1 can result in immuno-suppression similar to its human homolog. Studies suggest that although SIGNR1 has a similar binding specificity as DC-SIGN, its role is limited during murine M. tuberculosis infection (Wieland et al. 2007). Resistance to *M. tuberculosis* was impaired only in SIGNR3-deficient animals. SIGNR3 was expressed in lung phagocytes during infection, and interacted with *M. tuberculosis* bacilli and mycobacterial surface glycoconjugates to induce secretion of critical host defense inflammatory cytokines, including TNF. SIGNR3 signaling was dependent on an intracellular tyrosine-based motif and the tyrosine kinase Syk. Thus, the mouse DC-SIGN homolog SIGNR3 makes a unique contribution to protection of the host against a pulmonary bacterial pathogen (Tanne et al. 2009). The rat CD209b mediates the uptake of dextran or CPS14 within the rat splenic marginal zone, similar to SIGNR1. On microglia, rat CD209b also mediates the uptake of CPS14 of *S. pneumoniae*. Findings suggest that both rat CD209b and SIGNR1 on microglia mediate the SIGNR1 complement activation pathway against *S. pneumoniae*, and thereby plays an important role in the pathogenesis of pneumococcal meningitis (Park et al. 2009).

Marginal zone macrophages in the murine spleen play an important role in the capture of blood-borne pathogens and are viewed as an essential component of host defense against the development of pneumococcal sepsis. However, reports described the loss of marginal zone macrophages associated with the splenomegaly that follows a variety of viral and protozoal infections; this finding raises the question of whether these infected mice would become more susceptible to secondary pneumococcal infection. Contrary to expectations, Kirby et al. (2009) demonstrated that the normal requirement for SIGNR1$^+$ marginal zone macrophages to protect against a primary pneumococcal infection can be readily compensated for by activated red pulp macrophages under conditions of splenomegaly. SIGNR1 is crucial for the capture of *S.*

pneumoniae from blood. SIGNR1 is able to interact in vitro with the juxtaposing marginal zone B cell population, which is responsible for the production of early IgM response against the *S. pneumoniae*-epitope phosphorylcholine. Strikingly, SIGNR1-deficient mice display a reduction in the marginal zone B cell population. In addition, ex vivo B cell stimulation assays demonstrate a decrease in phosphorylcholine specificity in the splenic B cell population derived from SIGNR1-deficient mice, whereas the total IgM response was unaffected. Therefore, interaction of SIGNR1 expressed by marginal zone macrophages with marginal zone B cells is essential to early IgM responses against *S. pneumoniae* (Koppel et al. 2008; Saunders et al. 2009).

Inflammatory Bowel Disease: In context of the etiology of inflammatory bowel disease, there is a less defined function for C-type lectins. The CD209 gene is located in a region linked to inflammatory bowel disease (IBD). Though the CD209 functional polymorphism (rs4804803) has been associated to other inflammatory conditions, it does not seem to be influencing Crohn's disease susceptibility. However, it could be involved in the etiology or pathology of Ulcerative Colitis in HLA-DR3-positive individuals (Núñez et al. 2007). Saunders et al. (2010) demonstrated that mice deficient in SIGNR1 have reduced susceptibility to experimental colitis, with a reduction in the disease severity, colon damage, and levels of the proinflammatory cytokines IL-1β, TNF-α, and IL-6. SIGNR1$^{-/-}$ peritoneal macrophages, but not bone marrow-derived macrophages, had a specific defect in IL-1β and IL-18 production, but not other cytokines, in response to TLR4 ligand LPS. SIGNR1 was associated in the regulation of inflammation in a model of experimental colitis and is a critical innate factor in response to LPS.

36.9 Liver and Lymph Node Sinusoidal Endothelial Cell C-Type Lectin (LSECtin) (or CLEC4G or L-SIGN or CD209L)

36.9.1 Characterization and Localization

The liver is an organ with paradoxic immunologic properties and is known for its tolerant microenvironment, which holds important implications for hepatic diseases. Liver and lymph node sinusoidal endothelial cell C-Type lectin (LSECtin) (or CLEC4G or L-SIGN or CD209L) displays 77% amino acid identity with DC-SIGN, and is expressed on endothelial cells in lymph node sinuses, capillary endothelial cells in the placenta and on liver sinusoidal cells (LSECs) (Soilleux et al. 2000; Bashirova et al. 2001; Pohlmann et al. 2001; Engering et al. 2004). The LSECtin (CD209L), gene encodes a protein of 293 amino acids and maps to chromosome 19p13.3 adjacent to C-type lectin genes, CD23, DC-SIGN, and DC-SIGNR. The four genes form a tight cluster in an insert size of 105 kb and have analogous genomic structures. The LSECtin is a type II integral membrane protein of approximately 40 kDa in size with a single C-type lectin-like domain at the C-terminus, close in homology to DC-SIGNR, DC-SIGN, and CD23. LSECtin mRNA was expressed in liver and lymph node among 15 human tissues tested, intriguingly neither expressed on hematopoietic cell lines nor on monocyte-derived DCs. Colmenares et al. (2007) detected LSECtin expression in human peripheral blood and thymic dendritic cells. LSECtin is also detected in monocyte-derived macrophages and dendritic cells at RNA and protein level (Liu et al. 2004). LSECtin could also be detected in the MUTZ-3DC cell line at mRNA and protein level. Human liver revealed its presence in Kupffer cells coexpressing the myeloid marker CD68 (Domínguez-Soto et al. 2009). In vitro, IL-4 induces the expression of 3 LSECtin alternatively spliced isoforms, including a potentially soluble form (Δ2 isoform) and a shorter version of the prototypic molecule (Δ3/4 isoform).

Full-length porcine (p) CLEC4G (L-SIGN) cDNA encodes a type II transmembrane protein of 290 amino acids. The pCLEC4G gene has same gene structure as human and the predicted bovine, canis, mouse and rat CLEC4G genes with nine exons. The pCLEC4G mRNA expresses in liver, lymph node and spleen tissues. A series of sequential intermediate products of pCLEC4G pre-mRNA were also identified during splicing from pig liver. The chromosomal regions syntenic to the human cluster of genes CD23/CLEC4G/DC-SIGN/L-SIGN have been compared in mammalian species including primates, domesticated animal, rodents and opossum. The L-SIGN homologs do not exist in non-primates mammals (Huang and Peng 2009).

In the liver LSECs functions as liver-resident antigen presenting cells (Knolle and Gerken 2000) and is important in tolerance induction (Knolle and Limmer 2001). LSECs may mediate the clearance of antigens from the circulation in same manner as DCs (Bashirova et al. 2001; Karrar et al. 2007). DC-SIGN and L-SIGN (LSECtin) share a di-leucine motif and a cluster of three acidic amino acids in their cytoplasmic tails, which are known to be essential for antigen uptake (Bashirova et al. 2001; Engering et al. 2002). Recent studies with Ebola virus, Severe Acute Respiratory Syndrome (SARS) virus or antibodies against L-SIGN, clearly demonstrated that L-SIGN indeed is able to internalize antigens (Klimstra et al. 2003; Liu et al. 2004; Jeffers et al. 2004; Ludwig et al. 2004; Dakappagari et al. 2006). DC-SIGN transient expression in HEK293T is a useful model for investigating p38 MAPK pathway

triggered by hepatitis C virus glycoprotein E2, which may provide information for understanding cellular receptors-mediated signaling events and the viral pathogenesis (Chen et al. 2010).

36.9.2 Ligands of LSECtin

LSECtin binds to mannose, GlcNAc, and fucose in a Ca^{2+}-dependent manner but not to galactose (Liu et al. 2004). The DC-SIGN and DC-SIGNR (DC-SIGN/R) bind to high-mannose carbohydrates on a variety of viruses. In contrast, the related lectin LSECtin does not recognize mannose-rich glycans and interacts with a more restricted spectrum of viruses. LSECtin and DC-SIGNR, which are co-expressed by liver, lymph node and bone marrow sinusoidal endothelial cells, bind to soluble Ebola virus glycoprotein (EBOV-GP) with comparable affinities. Similarly, LSECtin, DC-SIGN and Langerin readily bound to soluble HIV-1 GP. However, only DC-SIGN captured HIV-1 particles, indicating that binding to soluble GP is not necessarily predictive of binding to virion-associated GP. Results reveal important differences between pathogen capture by DC-SIGN/R and LSECtin and hint towards different biological functions of these lectins (Gramberg et al. 2008). To compare the sugar and pathogen binding properties of LSECtin with those of related but more extensively characterized receptors, such as DC-SIGN, a soluble fragment of LSECtin consisting of the C-terminal CRD was expressed in bacteria and used to probe a glycan array and to characterize binding to oligosaccharide and glycoprotein ligands. LSECtin binds with high selectivity to glycoproteins terminating in GlcNAcβ1-2Man. Glycan analysis of the surface glycoprotein of Ebola virus reveals the presence of such truncated glycans, explaining the ability of LSECtin to facilitate infection by Ebola virus (Powlesland et al. 2008). A systematic study of DC-SIGN, DC-SIGNR and LSECtin suggested that 'agalactosylated N-glycans' are candidate ligands common to these lectins (Dominguez-Soto et al. 2010).

Polymorphisms of *CLEC4M* have been associated with predisposition for infection by severe acute respiratory syndrome coronavirus (SARS-CoV). LSECtin not only acts as an attachment factor for pathogens, but also recognizes "endogenous" activated T cells. The CD44 on Jurkat T cells is a candidate ligand of LSECtin. Moreover, LSECtin selectively bound CD44s, CD44v4 and CD44v8-10 by screening a series of typical CD44 isoforms. The interaction between CD44 and LSECtin is dependent on protein-glycan recognition. Findings indicate that CD44 is the first endogenous ligand of LSECtin, and that LSECtin is a ligand of CD44 (Tang et al. 2010).

36.9.3 Functions

LSECtin functions as a pathogen receptor, because its expression confers Ebola virus-binding capacity to leukemic cells. Sugar-binding studies indicate that LSECtin specifically recognizes N-acetyl-glucosamine, whereas no LSECtin binding to Mannan- or N-acetyl-galactosamine-containing matrices are observed. Antibody or ligand-mediated engagement triggers a rapid internalization of LSECtin, which is dependent on tyrosine and diglutamic-containing motifs within the cytoplasmic tail. Therefore, LSECtin is a pathogen-associated molecular pattern receptor in human myeloid cells. In addition, LSECtin participates in antigen uptake and internalization, and might be a suitable target in vaccination strategies (Colmenares et al. 2007). In liver, LSECtin specifically recognized activated T cells and negatively regulated their immune responses. In mice with T-cell-mediated acute liver injury, the lack of LSECtin accelerated the disease owing to an increased T-cell immune response, whereas the exogenous administration of recombinant LSECtin protein or plasmid ameliorated the disease via down-regulation of T-cell immunity. Results reveal that LSECtin is a novel regulator of T cells and expose a crucial mechanism for hepatic T-cell immune suppression, perhaps opening up a new approach for treatment of inflammatory diseases in the liver (Tang et al. 2009).

The L-SIGN (or LSECtin) is also expressed in human lung in type II alveolar cells and endothelial cells, both potential targets for SARS-CoV. Since, several other enveloped viruses including *Ebola* and *Sindbis* use CD209L as a portal of entry, and HIV and *hepatitis C virus* can bind to L-SIGN on cell membranes but do not use it to mediate virus entry, it appears that the large S glycoprotein of SARS-CoV may use L-SIGN, in addition to ACE2 in infection and pathogenesis (Jeffers et al. 2004). Capture of *Hepatitis C virus* (HCV) by L-SIGN results in *trans*-infection of hepatoma cells. L-SIGN polymorphism could influence the establishment and progression of HCV infection (Falkowska et al. 2006). There is no significant correlation between the genetic polymorphism of DC-SIGN's exon 4 and HCV infection susceptibility. 9/5 genotype distribution frequency of DC-SIGNR's exon 4 in patients with hepatitis C is significantly higher and may be associated with HCV infection susceptibility (Wang et al. 2007). The L-SIGN binds mycobacterial ManLAM but not AraLAM, suggesting that L-SIGN may bind *M. tuberculosis*. Binding assays suggest that L-SIGN interacts strongly with the (Man)2-ara and (Man)3-ara, but not with the man-ara, similar to DC-SIGN. It indicates that L-SIGN may be involved in the pathogenesis of *M. tuberculosis* infection and that the L-SIGN captures the infection through ManLAM and rapidly internalizes it to lysosomes. This shows that L-SIGN may be involved in the clearance of mycobacteria since L-SIGN is expressed on those sites in lymph nodes and

liver which are ideally suited for antigen capture and clearance. However, mycobacteria may target L-SIGN to invade those tissues. More research is necessary to investigate the specific role of L-SIGN in these infections.

36.9.4 Role in Pathology

LSECtin enhances infection driven by filovirus glycoproteins (GP) and the S protein of SARS coronavirus, but does not interact with HIV-1 and hepatitis C virus envelope proteins. Ligand binding to LSECtin was inhibited by EGTA but not by mannan, suggesting that LSECtin unlike DC-SIGN/R does not recognize high-mannose glycans on viral glycoproteins. LSECtin is N-linked glycosylated and glycosylation is required for cell surface expression. In nut-shell, LSECtin is an attachment factor that in conjunction with DC-SIGNR might concentrate viral pathogens in liver and lymph nodes (Gramberg et al. 2005). Li et al. (2008) genotyped 23 tagSNPs in 181 SARS patients and reported no significant association with disease predisposition. Genetic variations in this cluster also did not predict disease prognosis. However, Li et al. (2008) detected a population stratification of the VNTR alleles in a sample of 1145 Han Chinese collected from different parts of China. Li et al. (2008) indicated that the genetic predisposition allele was not found in this lectin gene cluster and population stratification might have caused the previous positive association.

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