Chapter 9
DC-SIGN in Infection and Immunity

Joris K. Sprokholt, Ronald J. Overmars, and Teunis B.H. Geijtenbeek

Abstract Dendritic cells (DCs) play a central role in the immune system by patrolling peripheral tissues to sample antigens to induce antigen-specific adaptive immune responses in lymphoid tissues. DCs express pattern recognition receptors such as toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and C-type lectin receptors (CLRs) to interact with pathogens for antigen presentation and immune activation. One of the CLRs involved in different processes of DC function is DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN). DC-SIGN recognition of pathogens leads to efficient internalization and processing of antigen for MHC class I and II presentation. In addition, triggering of DC-SIGN induces intracellular signaling that affects immune responses. Although DC-SIGN signaling by itself does not lead to activation of transcription factors such as NFκB, it greatly modifies signaling pathways induced by other receptors, including TLRs, RLRs, and interferon receptors. Modulation of signaling pathways by DC-SIGN tailors adaptive immune responses to different pathogens by driving specific T-helper cell responses. Intriguingly, DC-SIGN signaling depends on the carbohydrate structures present on pathogens as mannose structures induce very different signaling cascades than fucose structures, providing DCs with the plasticity to tailor immune responses to a diverse range of pathogens. Several pathogens however have evolved to subvert DC-SIGN functions for effective infection of DCs and efficient transmission to target cells. In this chapter we will discuss DC-SIGN structure, expression, and DC-SIGN functionality in shaping adaptive immune responses and immunopathogenesis.

Keywords Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) • Pathogen-recognition receptor (PRR) • Adhesion receptor • Antigen receptor • Adaptive immune responses • Innate signaling

J.K. Sprokholt • R.J. Overmars • T.B.H. Geijtenbeek (✉)
Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
e-mail: t.b.geijtenbeek@amc.uva.nl

S. Yamasaki (ed.), *C-Type Lectin Receptors in Immunity*,
DOI 10.1007/978-4-431-56015-9_9

9.1 Introduction

The primary function of dendritic cells (DCs) is patrolling peripheral tissues, sampling the environment for antigens, presentation of antigen to T cells, and shaping T-cell differentiation for effective immune responses and long-lasting immunity. DCs express an array of pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs) which recognize conserved molecular structures of pathogens. DCs use these receptors for pathogen binding, internalization, antigen presentation, and immune activation. In addition to pathogens, PRRs can recognize endogenous ligands to support key functions of DCs, including cellular contact with other (immune) cells. DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) (Geijtenbeek and Gringhuis 2009; Švajger et al. 2010), first described in 1992 in placenta (Curtis et al. 1992) and identified in 2000 as a DC-specific receptor (Geijtenbeek et al. 2000a), is a multifaceted CLR involved in primary functions of DCs.

DC-SIGN functions as an adhesion receptor involved in DC migration as well as DC-T-cell interactions. DC-SIGN mediates endothelial rolling for DC emigration from blood into peripheral tissue or secondary lymphoid organs (Geijtenbeek et al. 2000c). Once inside lymphoid tissues, DC-SIGN establishes the initial contact between DCs and T cells in order to scan MHC-peptide complexes by T-cell receptors (TCR) (Geijtenbeek et al. 2000a). Interestingly, DC-SIGN not only recognizes self-ligands but also interacts with different pathogens; and binding by DC-SIGN leads to internalization for antigen processing and presentation on MHC molecules (Engering et al. 2002a; Moris et al. 2004; Smith et al. 2007; Cambi et al. 2009). Pathogen recognition by DC-SIGN shapes adaptive immune responses by modulating signaling pathways induced by other receptors to tailor adaptive immune responses to different pathogens (Hovius et al. 2008; Geijtenbeek et al. 2009; Geijtenbeek and Gringhuis 2009; Gringhuis et al. 2009b, 2014a, b). However, DC-SIGN is also used by a diverse range of pathogens to subvert host immune mechanisms and establish productive infection (Geijtenbeek et al. 2000b; Trumpfheller et al. 2003; Ludwig et al. 2004; Mesman et al. 2014). These aspects of DC-SIGN functionality will be discussed in detail in this chapter.

9.2 DC-SIGN Structure Dictates Function

The molecular structure of DC-SIGN contains the blueprint for ligand specificity, endocytic potential, and signaling capacities. DC-SIGN is a type II transmembrane CLR with an intracellular N-terminus and an extracellular C-terminus (Cambi et al. 2009), similar to dectin-1 and mincle (Matsumoto et al. 1999; Ariizumi et al. 2000). The overall structure of DC-SIGN comprises a cytoplasmic tail

followed by a membrane domain, neck region, and carbohydrate recognition domain (CRD).

The cytoplasmic domain contains a di-leucine motif, which facilitates internalization (Engering et al. 2002a; Azad et al. 2008), and a tyrosine residue, which serves as a docking site for adapter proteins (Hodges et al. 2007; Gringhuis et al. 2009b). Several proteins have been proposed as adapter proteins for DC-SIGN, including leukemia-associated Rho guanine nucleotide exchange factor (LARG) and Ras homolog gene family member A (RhoA), but only leukocyte-specific protein 1 (LSP1) directly interacts with the cytoplasmic domain of DC-SIGN and serves as a docking site for additional proteins (Gringhuis et al. 2009b, 2014a; Hodges et al. 2007). The cytoplasmic domain is followed by a transmembrane domain of 18 amino acids and a neck domain, which contains 7.5 repeats of 23 amino acids that form α-helicases. These α-helicases contain hydrophobic residues which direct the CRD away from the cell surface and mediate the spontaneous formation of tetramers, thereby stabilizing DC-SIGN oligomers (Frisont et al. 2003). Additionally, the formation of tetramers enhances DC-SIGN specificity and avidity for repetitive structures (Frisont et al. 2003). Despite the formation of stable tetramers, sufficient flexibility is retained in the structure of DC-SIGN to adapt the CRDs for optimal binding of spatially distributed ligands (Leckband et al. 2011), which could explain why DC-SIGN is capable of binding a wide variety of ligands.

The CRD of DC-SIGN requires Ca^{2+} for structural stability as well as ligand binding (Geijtenbeek et al. 2000a; Cambi et al. 2009) and contains a conserved EPN motif that is essential for ligand binding (Geijtenbeek et al. 2002b; van Die et al. 2003). DC-SIGN is capable of binding mannose and fucose structures and to a lesser extent N-acetylglucosamine (GlcNAc) structures (Appelmelk et al. 2003; van Die et al. 2003; Meyer et al. 2005; Steeghs et al. 2006; van Liempt et al. 2006). Site-directed mutagenesis has revealed that mannose and fucose binding depends on the EPN motif and a valine residue in close proximity of the EPN motif (Geijtenbeek et al. 2002b; van Die et al. 2003). For GlcNAc structures, little is known about the amino acid residues of DC-SIGN involved in binding. In general, DC-SIGN affinity for GlcNAc structures is lower compared to fucose or mannose structures. It has been suggested that DC-SIGN binding to GlcNAc depends on the oligosaccharide backbone on which GlcNAc is presented, which could increase DC-SIGN avidity for GlcNAc structures (Steeghs et al. 2006).

In summary, the cytoplasmic domain of DC-SIGN is important for internalization (i.e., antigen uptake), signaling to shape immune responses, and intracellular trafficking for antigen processing; the neck domain is vital for protein stability and tetramer formation, and the CRD determines ligand specificity.

9.3 Cellular Expression of DC-SIGN Specifies Its Function

Cell-specific expression of DC-SIGN specifies its function, either by peripheral location, expression of relevant (signaling) proteins, or cell-intrinsic characteristics. DC-SIGN is expressed by distinct DC subsets in peripheral and lymphoid tissues but can also be found on certain macrophages (Geijtenbeek et al. 2000a, 2002a; Soilleux et al. 2001; Engering et al. 2002b, 2004; Granelli-Piperno et al. 2012).

Immature monocyte-derived DCs, differentiated using IL-4 and GM-CSF, express high levels of DC-SIGN, and this model has been used extensively to study DC-SIGN (Geijtenbeek et al. 2000a; Kwon et al. 2002; Engering et al. 2002a; Gringhuis et al. 2009b, 2010). However, DC-SIGN$^+$ DCs can also be found in peripheral tissues, including the skin, small and large intestine, and blood. Human skin harbors three distinct DC subsets: conventional CD1c$^+$ DCs, cross-presenting CD141$^+$ DCs, and monocyte-derived CD14$^+$ DCs (Haniffa et al. 2012; Mcgovern et al. 2014). Only CD14$^+$ DCs express DC-SIGN, and targeting this subset in human tissue using DC-SIGN-specific ligands has been investigated as an approach to induce tumor-specific immunity (Joshi et al. 2012; Unger et al. 2012). Although CD14$^+$ DCs are not classified as bona fide cross-presenting DCs, DC-SIGN-mediated endocytosis does lead to cross presentation on MHC class I molecules and activation of CD8$^+$ T cells (Unger et al. 2012; Fehres et al. 2015), highlighting that general statements about cell-intrinsic capacities do not necessarily hold true in the light of receptor-specific processes.

DC-SIGN$^+$ DCs in the large intestine are of clinical relevance in the sexual transmission of human immunodeficiency virus (HIV)-1, and possibly other pathogens, as HIV-1 targets DC-SIGN for efficient transmission to CD4$^+$ T cells (Geijtenbeek et al. 2000c; Kwon et al. 2002; Trumpfheller et al. 2003; Gurney et al. 2005). The same holds true for DC-SIGN$^+$ myeloid DCs in blood, which efficiently transmit HIV-1 to CD4$^+$ T cells (Engering et al. 2002b). In addition, DC-SIGN expression on blood DCs is important for endothelial rolling and DC migration into tissues by interacting with ICAM-2 on endothelial cells (Geijtenbeek et al. 2000c).

DC-SIGN$^+$ DCs in lymph nodes are likely to represent migrated DCs from peripheral tissues as well as resident DCs (Engering et al. 2004). DC-SIGN expression in lymph nodes is involved in establishing initial DC-T-cell interactions via DC-SIGN and intercellular adhesion molecule (ICAM)-3 expressed on naïve T cells (Geijtenbeek et al. 2000a).

In addition to DCs, DC-SIGN is expressed by decidual macrophages and Hofbauer cells in the placenta, where it could be involved in vertical transmission of HIV from mother to newborn (Geijtenbeek et al. 2001; Soilleux et al. 2001). DC-SIGN is also expressed by alveolar macrophages, which could be important during mycobacteria infections of the lung as DC-SIGN binds the mycobacterial cell wall component ManLAM (Soilleux et al. 2002; Geijtenbeek et al. 2003). However, little is known about the role of DC-SIGN in endocytosis, antigen processing, and signaling in macrophages. On a genomic level, DC-SIGN is encoded by *CD209*, located on

chromosome 19p13 and regulated by transcription factor PU.1 in combination with either MYB or RUNX3, which confine DC-SIGN expression to DCs and macrophages (Domínguez-Soto et al. 2005). Expression of DC-SIGN is enhanced by IL-4 via IL-4 receptor-mediated JAK-STAT6 signaling (Relloso et al. 2002). This signaling cascade is blocked by type I and II interferons (IFN) as they inhibit phosphorylation and nuclear translocation of STAT6 (Dickensheets et al. 1999; Svajger et al. 2010). This indicates that STAT6 could be an additional transcription factor for *CD209* or that STAT6 indirectly influences PU.1-MYB/RUNX3 transactivation activity. After translation, DC-SIGN is shuttled to the cell surface where it clusters into micro domains of 100–200 nm in diameter. These clusters are important for binding and internalization of virus particles (Cambi et al. 2005), probably by increasing avidity or by creating signaling scaffolds.

In conclusion, DC-SIGN is encoded by *CD209*, regulated by transcription factors PU.1, MYB, RUNX3, and possibly STAT6 and is expressed in micro domains on the cell membrane of different DC as well as macrophage subsets. The majority of studies investigating DC-SIGN are based on DCs, and it is therefore difficult to address whether DC-SIGN function is similar in macrophages without functional studies.

9.4 DC-SIGN Functionality Exposed

9.4.1 DC-SIGN Takes the Lead in Adhesion

DCs are professional antigen-presenting cells, which orchestrate adaptive immune responses by sampling antigens in the periphery and presenting peptides on MHC molecules to $CD4^+$ and $CD8^+$ T cells. One DC can present a vast number of different peptides, and initial DC-T-cell contact allows scanning of MHC-peptide complexes by TCRs. The initial contact is mediated by adhesion receptors, and DC-SIGN facilitates scanning by T cells by binding ICAM-3 on T cells, which is a N-linked glycosylated protein with high mannose oligosaccharides (Fig. 9.1b) (Bleijs et al. 2001; Geijtenbeek et al. 2000a). This transient contact is further stabilized via LFA-1-ICAM-1 interactions facilitating the formation of the immunological synapse for induction of T cell proliferation upon peptide recognition (Geijtenbeek et al. 2000a).

DC progenitors originate in the bone marrow and circulate the blood before migrating into peripheral tissues, where they sample antigen and home to secondary lymphoid organs. The migration from blood to tissue is a complex process involving leukocyte rolling, adhesion to endothelial cells, and transendothelial migration (Springer 1995). Endothelial cells lining blood and lymphatic vessels constitutively express the glycoprotein ICAM-2, which is crucial in leukocyte transendothelial migration. DC-SIGN functions as a DC-specific rolling receptor for ICAM-2 and mediates adhesion of DCs to the endothelium and subsequent transendothelial migration (Fig. 9.1a) (Geijtenbeek et al. 2000c, 2002a). In addition to ICAM molecules,

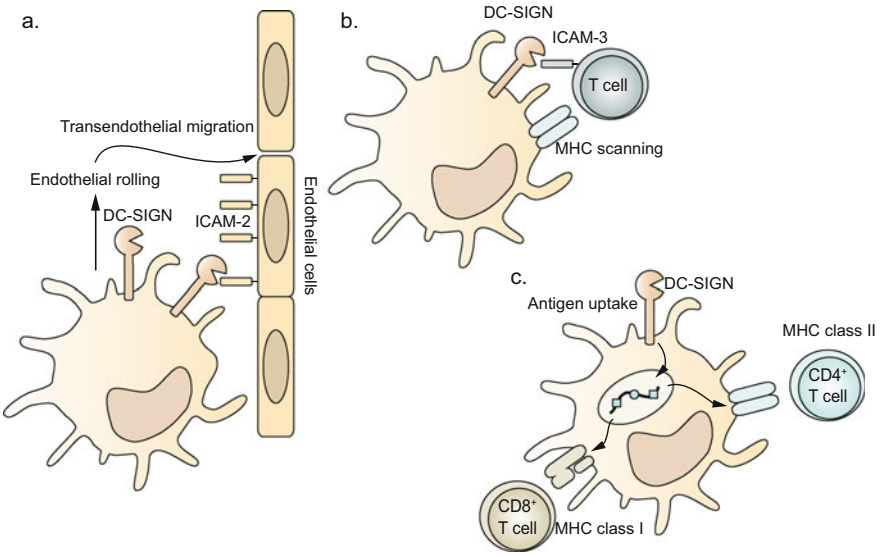

Fig. 9.1 DC-SIGN function in DC migration, T cell responses and antigen processing. (**a**) DC progenitors originate in the bone marrow and circulate the blood before migrating into peripheral tissues to sample antigens. DC migration from blood into tissue is receptor-dependent and requires interactions which can resist the shearing forces of blood circulation (Springer 1995). Endothelial cells express the glycoprotein ICAM-2 and adherence of DC-SIGN to ICAM-2 mediates DC rolling over endothelial surfaces. Once DCs have adhered to endothelial cells via LFA-1, DC-SIGN-ICAM-2 interactions mediate transendothelial migration of DCs to peripheral tissues. After sampling antigen, DCs migrate to secondary lymphoid structures for antigen presentation to T cells. (**b**) In secondary lymphoid structures, DCs present antigens on MHC class I or II molecules to T cells for the induction of adaptive immune responses. Initial DC-T-cell contact is antigen independent to allow scanning of MHC-peptide complexes by TCRs. DC-SIGN mediates this process by adhering to ICAM-3 on T cells. (**c**) DCs are professional antigen-presenting cells, which requires internalization and processing of antigens. Targeting DC-SIGN with either single molecules, pathogens, or carbohydrate-coated liposomes results in rapid internalization into endosomes. The endosomal cargo is then further routed to lysosomal compartments where antigen is processed for MHC class II presentation to $CD4^+$ T cells. Although the precise mechanism is unclear, endosomal cargo is probably also transported to the cytosol where it is loaded on MHC class I molecules for $CD8^+$ T cell presentation

DC-SIGN specifically interacts with β-integrin Mac-1 expressed by neutrophils due to neutrophil-specific glycosylation of Mac-1. This interaction facilitates DC-neutrophil clustering and DC maturation by activated neutrophils (van Gisbergen et al. 2005). Hence, DC-SIGN plays a central role in establishing cell-cell contact between DCs and other cells such as T cells, endothelial cells, and neutrophils to support DC migration, maturation, and induction of adaptive immune responses.

9.4.2 DC-SIGN Has Excellent Presentation Skills

A key characteristic of DCs is endocytosis of antigens and subsequent presentation of peptides on MHC class I molecules for $CD8^+$ cytotoxic T cell responses or on MHC class II molecules for the induction of $CD4^+$ T cell responses. Soluble antigens, intact pathogens, and individual molecules are captured by DC-SIGN and internalized via clathrin-coated pits (Cambi et al. 2009; Engering et al. 2002a). Endocytosis of DC-SIGN depends on the di-leucine motif in the cytoplasmic domain of DC-SIGN (Engering et al. 2002a; Azad et al. 2008). Upon internalization, DC-$SIGN^+$ endosomes are routed to the endo-lysosomal pathway for antigen processing and subsequent loading of peptides on MHC class II molecules for presentation to $CD4^+$ T cells (Fig. 9.1c) (Geijtenbeek et al. 2002a; Engering et al. 2002a; Schjetne et al. 2002). However, several pathogens that are captured by DC-SIGN are not targeted to lysosomal compartments. Instead, they are retained in (early) endosomes and remain infectious for extended periods of time, as has been shown for HIV and HCV (Geijtenbeek et al. 2000b; Cormier et al. 2004; Ludwig et al. 2004). This indicates that DC-SIGN-dependent internalization can lead to different intracellular routing pathways depending on the ligand. Multivalency of the carbohydrates or carbohydrate structures might be involved in the different internalization routes.

In addition to MHC class II presentation, DCs have the unique capacity to internalize and process antigen for cross-presentation on MHC class I molecules, which can occur via two mechanisms. Either by (1) transporting antigen from endosomes to the cytosol for proteasomal degradation and loading on MHC class I molecules in the ER or (2) by processing and loading of antigen on MHC class I molecules in endo-lysosomal compartments (Joffre et al. 2012). Targeting antigens to DC-SIGN leads to efficient cross presentation and induction of $CD8^+$ T cell responses using either monocyte-derived DCs and $CD14^+$ dermal DCs in human skin explants or in vivo using mice (Tacken et al. 2012; Unger et al. 2012; Fehres et al. 2015). Although it is unclear what the underlying mechanisms are for DC-SIGN-dependent cross presentation, these studies indicate that internalization of pathogens by DC-SIGN could lead to MHC class I presentation and $CD8^+$ T cell responses. Indeed, HIV-1 capture by DC-SIGN can lead to MHC class I presentation under specific circumstances (Moris et al. 2004).

Receptor-mediated endocytosis is a fundamental component in inducing antigen-specific immune responses and forms the basis for antigen uptake, processing, and presentation. DC-SIGN binds with high affinity to a wide variety of pathogens and mediates internalization and processing of pathogens for peptide presentation on MHC molecules. Therefore, DC-SIGN plays a central role in antigen-specific immune responses against numerous pathogens, which could be harnessed in vaccine development.

9.4.3 DC-SIGN Signaling Tailors Immune Responses

DC-SIGN is a multivalent receptor that interacts with mannose, fucose, and GlcNAc structures present on a diverse range of pathogens, including HIV-1, *Mycobacterium tuberculosis*, *Candida albicans,* and *Schistosoma mansoni*. Although DC-SIGN signaling does not directly lead to induction of immune responses, it greatly affects signaling induced by other PRRs such as TLRs and RLRs (Geijtenbeek et al. 2009; Geijtenbeek and Gringhuis 2009; Gringhuis et al. 2007, 2009b; Mesman et al. 2014). Interestingly, DC-SIGN signaling depends on the nature of the pathogen as mannose structures induce very different signaling cascades from fucose structures, which critically alters adaptive T cell responses (Geijtenbeek et al. 2003; Gringhuis et al. 2009b, 2014a, b; Geijtenbeek and Gringhuis 2009).

9.4.3.1 Mannose Signaling

Under homeostatic conditions, DC-SIGN is constitutively associated with a signaling complex consisting of LSP1, kinase suppressor of Ras (KSR)-1, connector enhancer of KSR (CNK), and serine/threonine kinase Raf-1 (Gringhuis et al. 2009b). Activation of DC-SIGN by mannose-containing structures or pathogens, including HIV-1, measles virus (MV), *Candida albicans,* and mycobacteria recruits the additional proteins LARG, RhoA, and GTP-Ras (Gringhuis et al. 2009b). These proteins induce a complex chain of events that ultimately leads to the phosphorylation, and thereby activation, of Raf-1 at Ser338 and Tyr340-341 by p21-activated kinases (PAKs) and Src kinases, respectively (Gringhuis et al. 2007). Activation of Raf-1 subsequently leads to the phosphorylation of NFκB subunit p65 at Ser276 (Gringhuis et al. 2007). A prerequisite for p65 phosphorylation by Raf-1 is prior activation of NFκB by others PRRs, such as TLR4, as DC-SIGN signaling by itself does not induce NFκB activation. Phosphorylated p65 by Raf-1 facilitates complex formation of p65 with CREB-binding protein (CBP) and p300, leading to acetylation of p65 (Chen et al. 2005; Gringhuis et al. 2007). Acetylation of p65 increases its DNA-binding affinity and transcriptional rate and prolongs nuclear activity (Chen et al. 2002). This results in increased transcription of *Il10*, *Il12a*, *Il12b*, and *Il6* genes, which are critical cytokines for skewing T_H differentiation (Fig. 9.2a) (Gringhuis et al. 2009b).

Raf-1 is known for its function in the Raf-MEK-ERK pathway. This pathway is involved in cell fate decisions such as cell growth, differentiation, and survival. However, binding of mannose structures to DC-SIGN leads to specific Raf-1 activation, without triggering ERK1/2 or MEK-1/2 (Wellbrock et al. 2004; Gringhuis et al. 2007). It is unclear why DC-SIGN-dependent Raf-1 activation does not lead to canonical ERK1/2-MEK1/2 signaling, but this is probably caused by the cellular location of Raf-1 during DC-SIGN signaling and the DC-SIGN-specific signaling complex that leads to Raf-1 activation. However, the lack of ERK1/2-MEK1/2 signaling by mannose structures could also be ligand-specific as other ligands of DC-SIGN, such as the tick saliva protein Salp15, induce MEK1/2

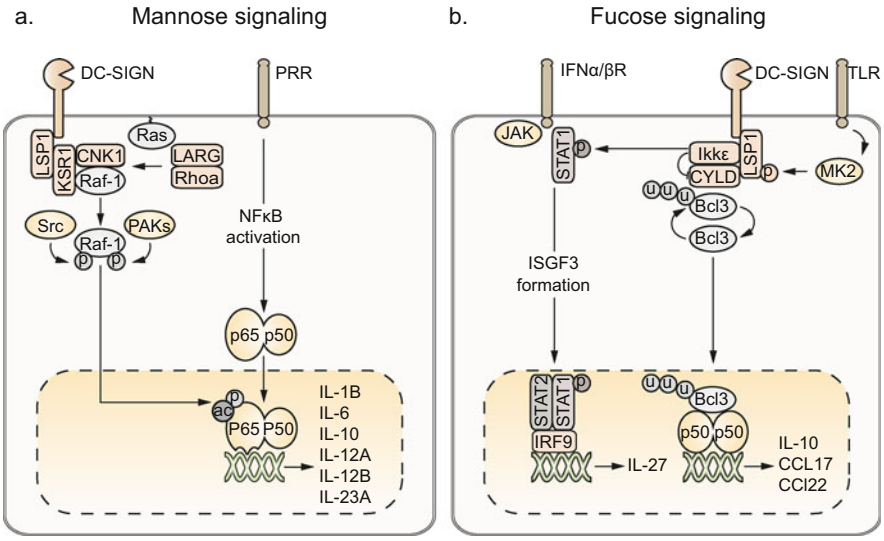

Fig. 9.2 DC-SIGN modulates intracellular signaling pathways. DC-SIGN is unable to induce gene expression on its own, but DC-SIGN signaling greatly affects signaling pathways induced by other receptors with a decisive outcome on adaptive immune responses. (**a**) DC-SIGN is under homeostatic conditions constitutively associated with the adapter molecule LSP1 in combination with KSR1, CNK1, and the kinase Raf-1. Triggering of DC-SIGN by mannose-containing pathogens recruits LARG, Rhoa, and GTP-Ras, which ultimately leads to the phosphorylation and activation of Raf-1 by PAKs and Src kinases. DC-SIGN-activated Raf-1 modulates NFκB signaling of other PRRs by inducing the phosphorylation and acetylation of NFκB subunit p65. This enhances the transcription of *IL-1B*, *IL-6*, *IL-10*, *IL-12A*, *IL-12B*, and *IL-23A* genes. These cytokines are crucial for the induction of specific T cell responses. (**b**) DC-SIGN triggering by fucose-containing pathogens leads to disassociation of KSR1-CNK1-Raf-1 from DC-SIGN-LSP1 and probably provides structural clearance for MK2-dependent phosphorylation of LSP1. MK2 is activated by TLR signaling, including TLR2, TLR3, and TLR4. Phosphorylation of LSP1 recruits the kinase IKKε and the deubiquitinase CYLD, which constantly removes ubiquitin chains from the noncanonical NFκB subunit Bcl3. IKKε and CYLD recruitment to LSP1 results in CYLD phosphorylation, decreasing its deubiquitinase activity and activating Bcl3. Ubiquitinated Bcl3 induces the formation of NFκB subunit p50 homodimers which decreases *IL1B*, *IL-6*, *IL-12A*, *IL-12B*, and *IL-23A* gene transcription and increases *IL-10*, *CCL17*, and *CCL22* transcription, thereby shifting the cytokine responses from a T_H1-skewing profile to a T_H2-inducing profile. In addition to CYLD-Bcl3, IKKε activation modulates IFNR JAK-STAT signaling, which is triggered by TLR-induced type I IFN. IKKε phosphorylates STAT1 at Ser708, leading to the formation of STAT1-STAT2 heterodimers in complex with IRF9 (ISGF3). ISGF3 binds to ISRE-containing genes including IL-27, a crucial cytokine in T_{FH} cell responses

but not ERK1/2 activation (Hovius et al. 2008); and triggering DC-SIGN using recombinant Hepatitis C protein E2 leads to both MEK and ERK activation (Zhao et al. 2013). In addition to distinct DC-SIGN signaling pathways induced by Salp15 and E2, MEK/ERK activation by these proteins could also be the results of triggering additional receptors. Indeed, both E2 and Salpt15 are known to interact with other receptors on DCs (Ogden and Tang 2015; Garg et al. 2010).

Thus, DC-SIGN is under homeostatic conditions in complex with LSP1-KSR1-CNK-Raf-1, and DC-SIGN activation by mannose-containing pathogens leads to the recruitment of LARG and RhoA, which activates kinase Raf-1, leading to p65 phosphorylation and acetylation. Acetylated p65 increases the transcription of *Il10*, *Il12a*, *Il12b*, and *Il6* genes, which are pivotal in adaptive T cell responses. Hence, DC-SIGN signaling plays a central role in innate and adaptive immune responses against mannose-containing pathogens. Notably, fucose binding results in a different composition of the LSP-1 signalosome, which will be discussed below.

9.4.3.2 Fucose Signaling

Mannose-induced DC-SIGN signaling depends on the LSP1-KSR1-CNK-Raf-1 signalosome. However, activation of DC-SIGN by fucose-containing pathogens, including *Schistosoma mansoni*, *Fasciola hepatica,* and *Helicobacter pylori* leads to disassociation of KSR1-CNK-Raf1 without affecting LSP1-DC-SIGN interaction. This allows mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 (MK2) to phosphorylate LPS1 at Ser204 and Ser252 (Gringhuis et al. 2014a). MK2 activation depends on MAP kinase p38, which is not induced by DC-SIGN signaling and requires additional PRR triggering. In particular, Gringhuis et al. (2014a) have shown that TLR2, TLR3, and TLR4 signaling activates p38-MK2 to phosphorylate LSP1 after fucose triggering of DC-SIGN. Although this study investigated TLRs, numerous cellular processes can activate p38-MK2, including cytokine receptor signaling, osmotic stress, and chemical stress (Roux and Blenis 2004). Whether these processes also lead to LSP1 phosphorylation by fucose-triggered DC-SIGN has not been investigated.

Phosphorylation of LSP1 at Ser252 results in recruitment of IkappaB kinase ε (IKKε) and the deubiquitinase CYLD to DC-SIGN. Under homeostatic conditions, CYLD prevents noncanonical NFκB subunit Bcl3 translocation to the nucleus by continuous removal of K63-linked polyubiquitin chains (Massoumi et al. 2006). However, fucose-DC-SIGN-induced complex formation of LSP1-IKKε-CYLD results in IKKε phosphorylation at Ser172 and subsequent phosphorylation of CYLD at Ser418. Phosphorylation of CYLD decreases its deubiquitinase activity, allowing Bcl3 activation and nuclear translocation (Fig. 9.2b) (Gringhuis et al. 2014a).

TLR4 activation by LPS normally leads to NFκB p50-p65 dimers, which bind to promoter sites in *IL6*, *IL10*, *IL12A*, *IL12B*, and *IL23A* genes (Ghosh and Hayden 2008). However, simultaneous triggering of DC-SIGN by fucose structures leads to Bcl3-induced p50 dimers with decreased transcriptional activity at *IL6*, *IL12A*, *IL12B*, and *IL23A* genes and similar transcriptional activity at *IL10* compared to p50-p65 heterodimers. In addition, Bcl3-p50-p50 complexes induce the expression of T_H2 attracting chemokines CCL17 and CCL22 (Gringhuis et al. 2014a). Therefore, cross talk between TLRs and DC-SIGN shifts the cytokine responses from a T_H1-skewing profile to a T_H2-inducing profile, which is crucial in clearing extracellular parasites like *Schistosoma mansoni* (Gringhuis et al. 2014a).

In addition to IKKε-CYLD-dependent Bcl3 activation by DC-SIGN, IKKε alters IFNα/β receptor (IFNR) signaling. TLR-induced IFN-β production leads to autocrine IFNR activation and JAK-STAT signaling, resulting in the formation of STAT1 homodimers, which bind to IFN-γ-activated sites (GAS) in specific genes (Decker et al. 2005). However, IKKε, activated by fucose-triggered DC-SIGN, phosphorylates STAT1 at Ser708, which leads to the formation of IFN-stimulated gene factor 3 (ISGF3), a protein complex consisting of STAT-1-STAT2 heterodimers and the DNA-binding unit IRF9 (González-Navajas et al. 2012; Gringhuis et al. 2014b). ISGF3 binds to IFN-stimulated response elements (ISRE) instead of GAS elements and thereby induces a different set of genes compared to STAT1 homodimers (Decker et al. 2005). In particular, ISGF3 activation leads to pronounced and prolonged expression of IFN-β as well as specific ISRE-dependent genes. ISRE-containing genes comprise important antiviral genes, including 29,59-oligoadenylate synthetase 1 and myxovirus resistance protein A (Takaoka and Yanai 2006), and cytokines such as IL-27, which is essential for follicular T-helper (T_{FH}) cell formation (Batten et al. 2010; Gringhuis et al. 2014b).

Fucose-specific DC-SIGN signaling not only modulates TLR signaling but also IFNR signaling to mount T_H2 and T_{FH} responses important for antibody-mediated immunity. This suggests that T_H2 cells are closely linked to T_{FH} responses as both cell types are induced simultaneously. As humoral immune responses are critical against viruses, parasites, and bacteria (Tangye et al. 2013), fucose-based strategies targeting DC-SIGN have the potential to be harnessed for vaccine development.

9.4.4 DC-SIGN Fine Tunes Adaptive Immunity

For the innate immune system, pathogens are basically a collection of pathogen-associated molecular patterns (PAMPs). Tailored immune responses require activation of different PRRs by these PAMPs to specify the invading pathogen. This demands cross talk of different PRR-induced signaling pathways to induce tailored adaptive immune responses by DCs. For example, *M. tuberculosis* infection induces T_H1 and T_H17 responses, which rely on IL-12 and IL-23 secretion by DCs (Khader et al. 2007). IL-12 is essential in inducing T_H1 differentiation and stimulation of DCs with *M. tuberculosis* leads to robust IL-12 secretion (Gringhuis et al. 2009b). Interestingly, blocking DC-SIGN or inhibition of Raf-1 decreases IL-12 production by *M. tuberculosis*-stimulated DCs (Gringhuis et al. 2009b), resulting in a shift from protective T_H1 to T_H2 (Gringhuis et al. 2009a). Furthermore, inhibiting DC-SIGN-Raf-1 signaling in *M. tuberculosis*-stimulated DCs also reduces IL-23 production (Gringhuis et al. 2009b), which is critical for T_H17-mediated *M. tuberculosis* responses (Khader et al. 2007). Although the direct effect of DC-SIGN-Raf-1 signaling on T_H17 responses has not been investigated, increased IL-23 production probably promotes T_H17 responses against *M. tuberculosis* (Fig. 9.3a). This indicates that DC-SIGN is crucial for *M. tuberculosis*-induced immune responses and possibly other mannose-containing pathogens.

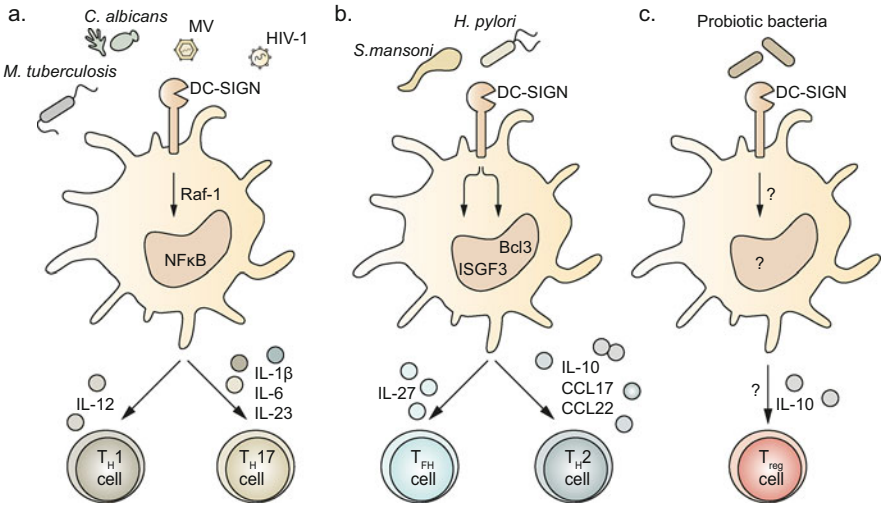

Fig. 9.3 DC-SIGN tailors adaptive immune responses. (**a**) Mannose-containing pathogens including MV, HIV-1, *C. albicans*, and *M. tuberculosis* induce DC-SIGN-dependent Raf-1 activation leading to phosphorylation and acetylation of NFκB subunit p65. This enhances the production of TLR-induced IL-12, which is crucial for the induction of T_H1 responses. In addition, Raf-1 activation leads to increased secretion of IL-1β, IL-6, and IL-23 which drive the formation of T_H17 responses. (**b**) Fucose-containing pathogens such as the parasite *S. mansoni* and the bacterium *H. pylori* induce DC-SIGN-dependent T_H2 and T_{FH} cell responses. Fucose-specific DC-SIGN signaling in combination with TLR activation induces Bcl3-dependent decrease of IL-1β, IL-6, IL-12, and IL-23 secretion while enhancing IL-10 secretion and production of T_H2-attracting chemokines CCL17 and CCL22. This drives the formation of T_H2 cells. Furthermore, fucose-specific DC-SIGN signaling modulates IFNR signaling to induce the production of IL-27, which is pivotal in T_{FH} formation. **c**. Probiotic bacteria like *L. reuteri* and *L. casei* are internalized by DCs via DC-SIGN. This leads to the secretion of IL-10 and the formation of T_{reg} cells. Although the formation of T_{reg} depends on DC-SIGN, it is unclear if *L. reuteri* and *L. casei* induce DC-SIGN-specific signaling pathways that differ from mannose or fucose induced signaling pathways

The clearance of extracellular pathogens depends on robust T_H2 responses (Allen and Sutherland 2014). The extracellular bacterium *H. pylori* induces T_H1 or T_H2 responses depending on the phase-variable expression of fucose structures (Bergman et al. 2004). Interestingly, only fucose-expressing *H. pylori* binds to DC-SIGN and induce T_H2 responses (Bergman et al. 2004) by inducing DC-SIGN-dependent Bcl3 activation (Gringhuis et al. 2014a). Activation of Bcl3 by DC-SIGN lowers pro-inflammatory cytokine secretion and increases IL-10 secretion in combination with increased production of T_H2-attracting chemokines CCL17 and CCL22 (Fig. 9.3b) (Gringhuis et al. 2014a). Whether other molecules important for T_H2 responses, such as OX40, are also induced by DC-SIGN-dependent Bcl3 activation has not been investigated (Gringhuis et al. 2014a). These studies indicate that the sole expression of fucose structures can completely shift the paradigm from T_H1 to T_H2 dominated immune responses, highlighting the importance of carbohydrates and CLRs in adaptive immunity.

The induction of regulatory T (T_{reg}) cells by probiotic bacteria, including *Lactobacillus reuteri* and *Lactobacillus casei*, has been linked to DC-SIGN-mediated uptake by DCs (Fig. 9.3c) (Smits et al. 2005). Although the presence of carbohydrate structures on *L. reuteri* and *L. casei* has not been investigated, the closely related bacterium *L. plantarum* expresses glycoproteins on the surface containing GlcNAcs (Fredriksen et al. 2012). DC-SIGN is known to interact with GlcNAcs, but it is unclear if this triggers DC-SIGN signaling (Steeghs et al. 2006; Zhang et al. 2006). Furthermore, the probiotic bacteria used by Federiksen et al. (2012) did not trigger any TLRs, which could also explain the induction of T_{reg} cells independent of DC-SIGN.

In addition to T-cell-driven immunity, humoral immune responses are pivotal in protection against many diseases. Humoral immune responses are induced in specific areas in secondary lymphoid organs called germinal centers (GC), where B cells differentiate into memory B cells and long-lived plasma cells. T_{FH} in GC play critical roles in regulating B cell differentiation and antibody isotype class switching. The differentiation and maintenance of T_{FH} by DCs is not fully understood but involves IL-6, IL-21, and IL-27 (Vogelzang et al. 2008; Batten et al. 2010; Nurieva et al. 2010; Gringhuis et al. 2014b). Interestingly, fucose-specific DC-SIGN signaling, in combination with TLR signaling, induces the production of IL-27 by DCs, which drives the formation of T_{FH} cells (Fig. 9.3b) (Gringhuis et al. 2014b). Furthermore, DC-SIGN-dependent IL-27-generated T_{FH} cells produce IL-21 and induce B cell class switching from IgM to IgG (Gringhuis et al. 2014b), indicating that DC-SIGN signaling by fucose-containing pathogens is crucial for humoral immune responses.

Hence, DC-SIGN shapes adaptive immune responses by directing T cell differentiation towards T_H1/T_H17 or T_H2/T_{FH} dominated responses, depending on the carbohydrate profile of pathogens and cross talk with other receptors, greatly enhancing the plasticity of DCs to tailor immune responses to a diverse range of pathogens.

9.5 DC-SIGN Tale from the Murine Perspective

Animal models have proven valuable in determining the overall outcome of molecular or cellular processes studied in vitro. The human DC-SIGN family consists of two receptors: DC-SIGN and L-SIGN, whereas mice have eight DC-SIGN homologs (SIGNR 1–8). However, all mouse homologs differ in glycan specificity, internalization capacity, innate signaling, and cell-specific expression, making mice unsuitable to study DC-SIGN functionality (Garcia-Vallejo and van Kooyk 2013; Soilleux et al. 2000; Bashirova et al. 2001; Powlesland et al. 2006; Park et al. 2001; Takahara et al. 2004; Tanne et al. 2009).

A transgenic mouse model has been created by expressing DC-SIGN under transcriptional control of the murine CD11c promoter to limit expression to DCs (Schaefer et al. 2008). Transgenic hSIGN mice express low levels of DC-SIGN,

are protected against *M. tuberculosis* infections compared to wild type mice, and in vivo targeting of DC-SIGN using antibody-antigen complexes enhances T-cell-mediated immune responses (Schaefer et al. 2008; Hesse et al. 2013). However, since cross talk with other PRRs is essential for DC-SIGN signaling, the question remains if hSIGN transgenic mice are suitable to study the full potential of DC-SIGN (Schaefer et al. 2008; Garcia-Vallejo and van Kooyk 2013).

9.6 DC-SIGN Picks Up the Wrong Hitchhikers

Pathogens are under constant pressure of the immune system for survival and have evolved intriguing ways to escape or prevent immune responses. Some pathogens have even hijacked immune components for efficient infection and dissemination in the host. In particular, HIV-1 uses DC-SIGN for efficient transmission to T cells. HIV-1 primarily infects T cells via CD4-CCR5/CXR4, which requires dissemination of the virus from the primary side of infection to secondary lymphoid tissues rich in $CD4^+$ T cells. As DCs constantly sample antigens in peripheral tissues for presentation to T cells, DCs form an ideal transport vehicle for HIV-1. Indeed, DC-SIGN binding to HIV-1 glycoprotein gp120 leads to effective internalization of HIV-1 by DCs (Geijtenbeek et al. 2000b; Turville et al. 2001; Engering et al. 2002b; Smith et al. 2007). Notably, HIV-1 is not routed for lysosomal degradation after DC-SIGN-mediated uptake but is retained in endosomes for several days (Geijtenbeek et al. 2000b). While stored, HIV-1 remains infectious and is released upon DC-T-cell interaction to infect $CD4^+$ T cells (Geijtenbeek et al. 2000b). In addition, DC-SIGN signaling is essential for productive infection of DCs by HIV-1. Release of HIV-1 ssRNA into the cytoplasm depends on HIV-1 binding to CD4 in combination with either CCR5 or CXR4, ultimately resulting in the integration of HIV-1 DNA into the DC genome. However, HIV-1 integration does not lead to productive transcription of HIV-1 DNA and requires TLR8 activation by HIV-1 ssRNA and DC-SIGN activation by gp120. HIV-1-triggered DC-SIGN signaling activates Raf-1, leading to the acetylation of p65 – after NFκB activation by TLR8 – which is essential for full-length transcription of HIV-1 (Gringhuis et al. 2010). Hence, HIV-1 hijacks DC-SIGN for productive infection of DCs and efficient transmission to T cells.

Induction of protective antiviral responses mediated by type I IFNs is paramount to limit viral infections. However, viruses have evolved ways to subvert host innate immunity by shielding replication complexes from detection, degrading essential host molecules, and inhibiting innate detection by activating specific signaling cascades (Ye et al. 2013). Measles virus (MV) is a highly contagious pathogen which infects $CD150^+$ DCs and T and B lymphocytes (Lemon et al. 2011; De Vries et al. 2012). MV is a negative stranded RNA virus, which replicates in the cytoplasm of infected cells and is prone for detection by cytosolic RIG-I-like receptors (RLRs). RLR activation is tightly regulated by continuous phosphorylation of caspase recruitment domains, and these require dephosphorylation by PP1

phosphatases for type I IFN induction (Wies et al. 2013). Intriguingly, MV induces DC-SIGN-dependent activation of Raf-1, which phosphorylates PP1 inhibitor I-1 and thereby prevents dephosphorylation of RLRs and type I IFN production, leading to enhanced infection of DCs (Mesman et al. 2014). Furthermore, the main target cells of dengue virus are DCs and multiple studies have shown that dengue virus depends on DC-SIGN for productive infection of DCs (Tassaneetrithep et al. 2003; Lozach et al. 2005). Whether dengue virus also activates DC-SIGN signaling to inhibit type I IFN induction in DCs has not been investigated.

These studies highlight that pathogens take advantage of the binding capacity of DC-SIGN in combination with specific DC-SIGN signaling for effective infection of DCs and transmission to target cells. Together with the migratory capacity of DCs, this activity makes DC-SIGN$^+$ DCs the ideal vehicle for viral dissemination.

9.7 Clinical Relevance and Therapeutic Potential

Single nucleotide polymorphisms (SNPs) can affect protein expression and function and can therefore be valuable tools to study the clinical relevance of molecular processes, which would otherwise be impossible in humans. Certain SNPs in CD209, the gene coding for DC-SIGN, have been identified that alter the pathogeneses of several diseases. For instance, DCSIGN1-366G is a variant of DC-SIGN with a SNP in the promoter sequence of CD209, which results in lower expression levels at the cell membrane. Individuals carrying the DCSIGN1-366G variant have lower incidence of dengue fever, which is in concordance with in vitro studies (Sakuntabhai et al. 2005; Lozach et al. 2005). In addition, DCSIGN1-366G is associated with protection against *M. tuberculosis*, as individuals carrying this variant have a lower incidence of lung cavitation (Vannberg et al. 2008). SNPs located in other noncoding parts of *CD209* have been implicated in the vertical transmission of HIV-1 from mother to child (Boily-Larouche et al. 2012; da Silva et al. 2012). SNP variants occurring in the neck region of DC-SIGN have been associated with increased vertical transmission. Molecular studies revealed that these variants increase HIV-1 binding and transmission to T cells, which could explain enhanced vertical transmission (Boily-Larouche et al. 2012). These studies emphasize the important role of DC-SIGN in HIV-1, *M. tuberculosis*, and dengue virus pathogenesis, which has spiked the interest to design prophylactics or carbohydrate-based therapies directed against DC-SIGN to prevent or treat disease (Alen et al. 2011; Varga et al. 2014). However, certain precaution should be considered before using DC-SIGN-inhibiting therapies as DC-SIGN is involved in basic functions of DCs.

DC-based vaccination is under intense interest to induce effective immunity against cancer and infectious diseases. Currently used DC therapies in the clinic are complex and require the isolation of monocytes (for monocyte-derived DCs) or DCs from blood, which are loaded with antigen ex vivo and injected back into patients (Kreutz et al. 2013). The capacity of DC-SIGN to internalize antigen for

MHC presentation and simultaneous modulation of cytokine profiles to direct T cell differentiation makes DC-SIGN an ideal receptor for vaccination strategies without the need to isolate DCs. For example, carbohydrate-coated liposomes filled with specific antigens targeting DC-SIGN induce strong $CD4^+$ and $CD8^+$ T cell responses directed against tumor antigens (Hesse et al. 2013; van Kooyk et al. 2013). Differential coating of liposomes with either mannose or fucose structures adds another level of refinement to these strategies to direct adaptive immune responses to the desired outcome. Whether similar successes can be achieved in humans needs to be addressed, but the enormous potential of these therapies requires quick action to investigate the efficiency of DC-SIGN-based therapies, particularly because these therapies can also be used as treatment for autoimmune diseases and infectious diseases (Kreutz et al. 2013).

9.8 Concluding Remarks

From the identification of DC-SIGN as a DC-specific receptor to the clarification of DC-SIGN-induced signaling cascades, DC-SIGN has revealed many aspects of DC functionality and the importance of CLRs and carbohydrates in immune responses. DC-SIGN contributes to basic functions of DCs such as DC migration from blood into tissues and establishing cellular interaction with other immune cells (Geijtenbeek et al. 2000a, b; Bleijs et al. 2001; van Gisbergen et al. 2005). Unraveling DC-SIGN signaling has greatly contributed to our understanding of cross talk between different receptors on a molecular level with great effects on adaptive immune responses. DC-SIGN cross talk with other receptors provides DCs with the plasticity to mount specific immune responses to a wide variety of pathogens (Gringhuis et al. 2007, 2009b, 2014a, b; Hovius et al. 2008; Geijtenbeek and Gringhuis 2009). However, there is still much to discover. Although DC-SIGN signaling in the light of mannose and fucose structures is becoming clear, GlcNAc structures might induce yet another signaling cascade, which could affect T_{reg} responses induced by probiotic bacteria (Smits et al. 2005). In addition to IL-27, fucose-specific DC-SIGN formation of ISGF3 induces prolonged expression of type I interferon and antiviral interferon-stimulated genes (Gringhuis et al. 2014b), thereby inducing a highly antiviral state in DCs. Whether this indeed limits viral replication in DCs remains to be investigated, but this mechanism could be used to decrease viral infections in patients. The antigen routing properties of DC-SIGN together with its strong affinity for different antigens makes it an ideal receptor for targeted vaccines strategies to induce long-lasting immunity (Engering et al. 2002a; Cambi et al. 2009; Hesse et al. 2013; van Kooyk et al. 2013). This, in combination with the modulating effects of DC-SIGN signaling on adaptive immune responses, provides researchers with an extended toolbox to develop effective vaccines against multiple diseases, including cancer, autoimmune diseases, and infectious diseases.

References

Alen MMF, de Burghgraeve T, Kaptein SJF et al (2011) Broad antiviral activity of carbohydrate-binding agents against the four serotypes of dengue virus in monocyte-derived dendritic cells. PLoS One. doi:10.1371/journal.pone.0021658

Allen JE, Sutherland TE (2014) Host protective roles of type 2 immunity: parasite killing and tissue repair, flip sides of the same coin. Semin Immunol 26:329–340. doi:10.1016/j.smim.2014.06.003

Appelmelk BJ, van Die I, van Vliet SJ et al (2003) Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. J Immunol 170:1635–1639. doi:10.4049/jimmunol.170.4.1635

Ariizumi K, Shen GL, Shikano S et al (2000) Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. J Biol Chem 275:20157–20167. doi:10.1074/jbc.M909512199

Azad AK, Torrelles JB, Schlesinger LS (2008) Mutation in the DC-SIGN cytoplasmic triacidic cluster motif markedly attenuates receptor activity for phagocytosis and endocytosis of mannose-containing ligands by human myeloid cells. J Leukoc Biol 84:1594–1603. doi:10.1189/jlb.0308192

Bashirova AA, Geijtenbeek TB, van Duijnhoven GC et al (2001) A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. J Exp Med 193:671–678. doi:10.1084/jem.193.6.671

Batten M, Ramamoorthi N, Kljavin NM et al (2010) IL-27 supports germinal center function by enhancing IL-21 production and the function of T follicular helper cells. J Exp Med 207:2895–2906. doi:10.1084/jem.20100064

Bergman MP, Engering A, Smits HH et al (2004) Helicobacter pylori modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. J Exp Med 200:979–990. doi:10.1084/jem.20041061

Bleijs DA, Geijtenbeek TBH, Figdor CG, Van Kooyk Y (2001) DC-SIGN and LFA-1: a battle for ligand. Trends Immunol 22:457–463. doi:10.1016/S1471-4906(01)01974-3

Boily-Larouche G, Milev MP, Zijenah LS et al (2012) Naturally-occurring genetic variants in human DC-SIGN increase HIV-1 capture, cell-transfer and risk of mother-to-child transmission. PLoS One. doi:10.1371/journal.pone.0040706

Cambi A, Koopman M, Figdor C (2005) How C-type lectins detect pathogens. Cell Microbiol 7:481–488. doi:10.1111/j.1462-5822.2005.00506.x

Cambi A, Beeren I, Joosten B et al (2009) The C-type lectin DC-SIGN internalizes soluble antigens and HIV-1 virions via a clathrin-dependent mechanism. Eur J Immunol 39:1923–1928. doi:10.1002/eji.200939351

Chen LF, Mu Y, Greene WC (2002) Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-??B. EMBO J 21:6539–6548. doi:10.1093/emboj/cdf660

Chen L, Williams SA, Mu Y et al (2005) NF- κ B RelA phosphorylation regulates RelA acetylation NF- □ B RelA phosphorylation regulates RelA acetylation †. Mol Cell Biol 25:7966–7975. doi:10.1128/MCB.25.18.7966

Cormier EG, Durso RJ, Tsamis F et al (2004) L-SIGN (CD209L) and DC-SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus Emmanuel. Proc Natl Acad Sci U S A 101:14067–14072. doi:10.1073pnas.0405695101

Curtis BM, Scharnowske S, Watson AJ (1992) Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120. Proc Natl Acad Sci U S A 89:8356–8360. doi:10.1073/pnas.89.17.8356

Da Silva RC, Segat L, Zanin V et al (2012) Polymorphisms in DC-SIGN and L-SIGN genes are associated with HIV-1 vertical transmission in a Northeastern Brazilian population. Hum Immunol 73:1159–1165. doi:10.1016/j.humimm.2012.07.338

De Vries RD, Mesman AW, Geijtenbeek TBH et al (2012) The pathogenesis of measles. Curr Opin Virol 2:248–255. doi:10.1016/j.coviro.2012.03.005

Decker T, Müller M, Stockinger S (2005) The yin and yang of type I interferon activity in bacterial infection. Nat Rev Immunol 5:675–687. doi:10.1038/nri1684

Dickensheets HL, Venkataraman C, Schindler U, Donnelly RP (1999) Interferons inhibit activation of STAT6 by interleukin 4 in human monocytes by inducing SOCS-1 gene expression. Proc Natl Acad Sci U S A 96:10800–10805. doi:10.1073/pnas.96.19.10800

Domínguez-Soto A, Puig-Kröger A, Vega MA, Corbí AL (2005) PU.1 regulates the tissue-specific expression of dendritic cell-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin. J Biol Chem 280:33123–33131. doi:10.1074/jbc.M503401200

Engering A, Geijtenbeek TBH, van Vliet SJ et al (2002a) The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. J Immunol 168:2118–2126. doi:10.4049/jimmunol.168.5.2118

Engering A, Van Vliet SJ, Geijtenbeek TBH, Van Kooyk Y (2002b) Subset of DC-SIGN+ dendritic cells in human blood transmits HIV-1 to T lymphocytes. Blood 100:1780–1786. doi:10.1182/blood-2001-12-0179

Engering A, van Vliet SJ, Hebeda K et al (2004) Dynamic populations of dendritic cell-specific ICAM-3 grabbing nonintegrin-positive immature dendritic cells and liver/lymph node-specific ICAM-3 grabbing nonintegrin-positive endothelial cells in the outer zones of the paracortex of human lymph nodes. Am J Pathol 164:1587–1595. doi:10.1016/S0002-9440(10)63717-0

Fehres CM, van Beelen AJ, Bruijns SCM et al (2015) In-situ delivery of antigen to DC-SIGN+ CD14+ dermal dendritic cells results in enhanced CD8+ T cell responses. J Invest Dermatol. doi:10.1038/jid.2015.152

Fredriksen L, Mathiesen G, Moen A et al (2012) The major autolysin Acm2 from Lactobacillus plantarum undergoes cytoplasmic O-glycosylation. J Bacteriol 194:325–333. doi:10.1128/JB.06314-11

Frisont N, Taylor ME, Soilleux E et al (2003) Oligolysine-based oligosaccharide clusters: selective recognition and endocytosis by the mannose receptor and dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin. J Biol Chem 278:23922–23929. doi:10.1074/jbc.M302483200

Garcia-Vallejo JJ, van Kooyk Y (2013) The physiological role of DC-SIGN: a tale of mice and men. Trends Immunol 34:482–486. doi:10.1016/j.it.2013.03.001

Garg R, Juncadella IJ, Ramamoorthi N et al (2010) Cutting edge: CD4 is the receptor for the tick saliva immunosuppressor, Salp15. J Immunol 9:1–14. doi:10.1111/j.1600-6143.2008.02497.x.Plasma

Geijtenbeek TBH, Gringhuis SI (2009) Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol 9:465–479. doi:10.1038/nri2569

Geijtenbeek TB, Torensma R, van Vliet SJ et al (2000a) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 100:575–585

Geijtenbeek TBH, Kwon DS, Torensma R et al (2000b) DC-SIGN, a dendritic cell – specific HIV-1-binding protein that enhances trans -infection of T cells. Cell 100:587–597

Geijtenbeek TB, Krooshoop DJ, Bleijs DA et al (2000c) DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. Nat Immunol 1:353–357. doi:10.1038/79815

Geijtenbeek TBH, Van Vliet SJ, Van Duijnhoven GCF et al (2001) DC-SIGN, a dentritic cell-specific HIV-1 receptor present in placenta that infects T cells in trans – a review. Placenta 22:19–23. doi:10.1053/plac.2001.0674

Geijtenbeek TBH, Engering A, Van Kooyk Y (2002a) DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. J Leukoc Biol 71:921–931

Geijtenbeek TBH, Van Duijnhoven GCF, Van Vliet SJ et al (2002b) Identification of different binding sites in the dendritic cell-specific receptor DC-SIGN for intercellular adhesion molecule 3 and HIV-1. J Biol Chem 277:11314–11320. doi:10.1074/jbc.M111532200

Geijtenbeek TBH, Van Vliet SJ, Koppel EA et al (2003) Mycobacteria target DC-SIGN to suppress dendritic cell function. J Exp Med 197:7–17. doi:10.1084/jem.20021229

Geijtenbeek TBH, den Dunnen J, Gringhuis SI (2009) Pathogen recognition by DC-SIGN shapes adaptive immunity. Future Microbiol 4:879–890. doi:10.2217/fmb.09.51

Ghosh S, Hayden MS (2008) New regulators of NF-kappaB in inflammation. Nat Rev Immunol 8: 837–848. doi:10.1038/nri2423

González-Navajas JM, Lee J, David M, Raz E (2012) Immunomodulatory functions of type I interferons. Changes 29:997–1003. doi:10.1016/j.biotechadv.2011.08.021.Secreted

Granelli-Piperno A, Pritsker A, Pack M et al (2012) Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin/CD209 is abundant on macrophages in the normal human lymph node and is not required for dendritic cell stimulation of the mixed leukocyte reaction. J Immunol 127:358–366. doi:10.1016/j.jsbmb.2011.07.002.Identification

Gringhuis SI, den Dunnen J, Litjens M et al (2007) C-type lectin DC-SIGN modulates toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-κB. Immunity 26:605–616. doi:10.1016/j.immuni.2007.03.012

Gringhuis SI, den Dunnen J, Litjens et al (2009a) Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. Nat Immunol 10(2): 203–213, http://doi.org/10.1038/ni.1692

Gringhuis SI, den Dunnen J, Litjens M et al (2009b) Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to mycobacterium tuberculosis, HIV-1 and helicobacter pylori. Nat Immunol 10:1081–1088. doi:10.1038/ni.1778

Gringhuis SI, van der Vlist M, van den Berg LM et al (2010) HIV-1 exploits innate signaling by TLR8 and DC-SIGN for productive infection of dendritic cells. Nat Immunol 11:419–426. doi:10.1038/ni.1858

Gringhuis SI, Kaptein TM, Wevers BA et al (2014a) Fucose-specific DC-SIGN signalling directs T helper cell type-2 responses via IKKε- and CYLD-dependent Bcl3 activation. Nat Commun 5:3898. doi:10.1038/ncomms4898

Gringhuis SI, Kaptein TM, Wevers BA et al (2014b) Fucose-based PAMPs prime dendritic cells for follicular T helper cell polarization via DC-SIGN-dependent IL-27 production. Nat Commun 5:5074. doi:10.1038/ncomms6074

Gurney KB, Elliott J, Nassanian H et al (2005) Binding and transfer of human immunodeficiency virus by DC-SIGN + cells in human rectal mucosa. J Virol 79:5762–5773. doi:10.1128/JVI.79.9.5762

Haniffa M, Shin A, Bigley V et al (2012) Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. Immunity 37:60–73. doi:10.1016/j.immuni.2012.04.012

Hesse C, Ginter W, Förg T et al (2013) In vivo targeting of human DC-SIGN drastically enhances CD8+ T-cell-mediated protective immunity. Eur J Immunol 43:2543–2553. doi:10.1002/eji.201343429

Hodges A, Sharrocks K, Edelmann M et al (2007) Activation of the lectin DC-SIGN induces an immature dendritic cell phenotype triggering Rho-GTPase activity required for HIV-1 replication. Nat Immunol 8:569–577. doi:10.1038/ni1470

Hovius JWR, De Jong M a WP, Den Dunnen J et al (2008) Salp15 binding to DC-SIGN inhibits cytokine expression by impairing both nucleosome remodeling and mRNA stabilization. PLoS Pathog. doi:10.1371/journal.ppat.0040031

Joffre OP, Segura E, Savina A, Amigorena S (2012) Cross-presentation by dendritic cells. Nat Rev Immunol 12:557–569. doi:10.1038/nri3254

Joshi MD, Unger WJ, Storm G et al (2012) Targeting tumor antigens to dendritic cells using particulate carriers. J Control Release 161:25–37. doi:10.1016/j.jconrel.2012.05.010

Khader SA, Bell GK, Pearl JE et al (2007) IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during mycobacterium tuberculosis challenge. Nat Immunol 8:369–377. doi:10.1038/ni1449

Kreutz M, Tacken PJ, Figdor CG (2013) Targeting dendritic cells – why bother? Blood 121: 2836–2844. doi:10.1182/blood-2012-09-452078

Kwon DS, Gregorio G, Bitton N et al (2002) DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. Immunity 16:135–144. doi:10.1016/S1074-7613(02)00259-5

Leckband DE, Menon S, Rosenberg K et al (2011) Geometry and adhesion of extracellular domains of DC-SIGNR neck length variants analyzed by force-distance measurements. Biochemistry 50:6125–6132. doi:10.1021/bi2003444

Lemon K, de Vries RD, Mesman AW et al (2011) Early target cells of measles virus after aerosol infection of non-human primates. PLoS Pathog. doi:10.1371/journal.ppat.1001263

Lozach P-Y, Burleigh L, Staropoli I et al (2005) Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. J Biol Chem 280:23698–23708. doi:10.1074/jbc.M504337200

Ludwig IS, Lekkerkerker AN, Depla E et al (2004) Hepatitis C virus targets DC-SIGN and L-SIGN to escape lysosomal degradation. J Virol 78:8322–8332. doi:10.1128/JVI.78.15.8322-8332.2004

Massoumi R, Chmielarska K, Hennecke K et al (2006) Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-κB signaling. Cell 125:665–677. doi:10.1016/j.cell.2006.03.041

Matsumoto M, Tanaka T, Kaisho T et al (1999) A novel LPS-inducible C-type lectin is a transcriptional target of NF-IL6 in macrophages. J Immunol 163:5039–5048, doi: ji_v163n9p5039 [pii]

Mcgovern N, Schlitzer A, Gunawan M et al (2014) Human dermal CD14 + cells are a transient population of monocyte-derived macrophages. Immunity 41:465–477. doi:10.1016/j.immuni.2014.08.006

Mesman AW, Zijlstra-Willems EM, Kaptein TM et al (2014) Measles virus suppresses RIG-I-like receptor activation in dendritic cells via DC-SIGN-mediated inhibition of PP1 phosphatases. Cell Host Microbe 16:31–42. doi:10.1016/j.chom.2014.06.008

Meyer S, Van Liempt E, Imberty A et al (2005) DC-SIGN mediates binding of dendritic cells to authentic pseudo-Lewis Y glycolipids of Schistosoma mansoni cercariae, the first parasite-specific ligand of DC-SIGN. J Biol Chem 280:37349–37359. doi:10.1074/jbc.M507100200

Moris A, Nobile C, Buseyne F et al (2004) DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. Blood 103:2648–2654. doi:10.1182/blood-2003-07-2532

Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, Wang Y-H, Dong C (2010) Bcl6 mediates the development of t follicular helper cells. Science 9:1–14. doi:10.1111/j.1600-6143.2008.02497.x.Plasma

Ogden SC, Tang H (2015) The missing pieces of the HCV entry puzzle. Futur Virol 10:415–428. doi:10.2217/FVL.15.12

Park CG, Takahara K, Umemoto E et al (2001) Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN. Int Immunol 13:1283–1290. doi:10.1093/intimm/13.10.1283

Powlesland AS, Ward EM, Sadhu SK et al (2006) Widely divergent biochemical properties of the complete set of mouse DC-SIGN-related proteins. J Biol Chem 281:20440–20449. doi:10.1074/jbc.M601925200

Relloso M, Puig-Kröger A, Pello OM et al (2002) DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-beta, and anti-inflammatory agents. J Immunol 168:2634–2643. doi:10.4049/jimmunol.168.6.2634

Roux PP, Blenis J (2004) ERK and p38 MAPK-activated protein kinases : a family of protein kinases with diverse biological functions ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 68: 320–344. doi:10.1128/MMBR.68.2.320

Sakuntabhai A, Turbpaiboon C, Casadémont I et al (2005) A variant in the CD209 promoter is associated with severity of dengue disease. Nat Genet 37:507–513. doi:10.1038/ng1550

Schaefer M, Reiling N, Fessler C et al (2008) Decreased pathology and prolonged survival of human DC-SIGN transgenic mice during mycobacterial infection. J Immunol 180:6836–6845, doi: 180/10/6836 [pii]

Schjetne KW, Thompson KM, Aarvak T et al (2002) A mouse C k -speci ® c T cell clone indicates that DC-SIGN is an ef ® cient target for antibody-mediated delivery of T cell epitopes for MHC class II presentation. Int Immunol 14:1423–1430

Smith AL, Ganesh L, Leung K et al (2007) Leukocyte-specific protein 1 interacts with DC-SIGN and mediates transport of HIV to the proteasome in dendritic cells. J Exp Med 204:421–430. doi:10.1084/jem.20061604

Smits HH, Engering A, Van Der Kleij D et al (2005) Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 115:1260–1267. doi:10.1016/j.jaci.2005.03.036

Soilleux EJ, Barten R, Trowsdale J (2000) DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. J Immunol 165:2937–2942. doi:10.4049/jimmunol.165.6.2937

Soilleux EJ, Morris LS, Lee B et al (2001) Placental expression of DC-SIGN may mediate intrauterine vertical transmission of HIV. J Pathol 195:586–592. doi:10.1002/path.1026

Soilleux EJ, Morris LS, Leslie G et al (2002) Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro. J Leukoc Biol 71:445–457

Springer TA (1995) Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu Rev Physiol 57:827–872

Steeghs L, van Vliet SJ, Uronen-Hansson H et al (2006) Neisseria meningitidis expressing IgtB lipopolysaccharide targets DC-SIGN and modulates dendritic cell function. Cell Microbiol 8: 316–325. doi:10.1111/j.1462-5822.2005.00623.x

Svajger U, Anderluh M, Jeras M, Obermajer N (2010) C-type lectin DC-SIGN: an adhesion, signalling and antigen-uptake molecule that guides dendritic cells in immunity. Cell Signal 22: 1397–1405. doi:10.1016/j.cellsig.2010.03.018

Tacken PJ, De Vries IJM, Gijzen K et al (2012) Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti – DC-SIGN antibody effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a hum. Pharmacia 106:1278–1285. doi:10.1182/blood-2005-01-0318

Takahara K, Yashima Y, Omatsu Y, Yoshida H, Kimura Y, Kang YS, Steinman RM, Park CG, Inaba K (2004) Functional comparison of the mouse DC-SIGN, SIGNR1, SIGNR3 and Langerin, C-type lectins. Int Immunol 16(6):819–829. doi:10.1093/intimm/dxh084

Takaoka A, Yanai H (2006) Interferon signalling network in innate defence. Cell Microbiol 8: 907–922. doi:10.1111/j.1462-5822.2006.00716.x

Tangye SG, Ma CS, Brink R, Deenick EK (2013) The good, the bad and the ugly – TFH cells in human health and disease. Nat Rev Immunol 13:412–426. doi:10.1038/nri3447

Tanne A, Ma B, Boudou F, Tailleux L, Botella H, Badell E, Levillain F, Taylor ME, Drickamer K, Nigou J, Dobos KM, Puzo G, Vestweber D, Wild MK, Marcinko M, Sobieszczuk P, Stewart L, Lebus D, Gicquel B, Neyrolles O (2009) A murine DC-SIGN homologue contributes to early host defense against Mycobacterium tuberculosis. J Exp Med 206(10):2205–2220. doi:10.1084/jem.20090188

Tassaneetrithep B, Burgess TH, Granelli-Piperno A et al (2003) DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 197:823–829. doi:10.1084/jem.20021840

Trumpfheller C, Park CG, Finke J et al (2003) Cell type-dependent retention and transmission of HIV-1 by DC-SIGN. Int Immunol 15:289–298. doi:10.1093/intimm/dxg030

Turville SG, Arthos J, Donald KM et al (2001) HIV gp120 receptors on human dendritic cells. Blood 98:2482–2488. doi:10.1182/blood.V98.8.2482

Unger WWJ, Van Beelen AJ, Bruijns SC et al (2012) Glycan-modified liposomes boost CD4 + and CD8 + T-cell responses by targeting DC-SIGN on dendritic cells. J Control Release 160:88–95. doi:10.1016/j.jconrel.2012.02.007

Van Die I, van Vliet SJ, Nyame AK et al (2003) The dendritic cell-specific C-type lectin DC-SIGN is a receptor for Schistosoma mansoni egg antigens and recognizes the glycan antigen Lewis x. Glycobiology 13:471–478. doi:10.1093/glycob/cwg052

Van Gisbergen KPJM, Sanchez-Hernandez M, Geijtenbeek TBH, van Kooyk Y (2005) Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. J Exp Med 201:1281–1292. doi:10.1084/jem.20041276

Van Kooyk Y, Unger WWJ, Fehres CM et al (2013) Glycan-based DC-SIGN targeting vaccines to enhance antigen cross-presentation. Mol Immunol 55:143–145. doi:10.1016/j.molimm.2012.10.031

Van Liempt E, Bank CMC, Mehta P et al (2006) Specificity of DC-SIGN for mannose- and fucose-containing glycans. FEBS Lett 580:6123–6131. doi:10.1016/j.febslet.2006.10.009

Vannberg FO, Chapman SJ, Khor CC et al (2008) CD209 genetic polymorphism and tuberculosis disease. PLoS One 3:1–6. doi:10.1371/journal.pone.0001388

Varga N, Sutkeviciute I, Ribeiro-Viana R et al (2014) A multivalent inhibitor of the DC-SIGN dependent uptake of HIV-1 and Dengue virus. Biomaterials 35:4175–4184. doi:10.1016/j.biomaterials.2014.01.014

Vogelzang A, McGuire HM, Yu D et al (2008) A fundamental role for interleukin-21 in the generation of T follicular helper cells. Immunity 29:127–137. doi:10.1016/j.immuni.2008.06.001

Wellbrock C, Karasarides M, Marais R (2004) The RAF proteins take centre stage. Nat Rev Mol Cell Biol 5:875–885. doi:10.1038/nrm1498

Wies E, Wang MK, Maharaj NP et al (2013) Dephosphorylation of the RNA sensors RIG-I and MDA5 by the phosphatase PP1 is essential for innate immune signaling. Immunity 38:437–449. doi:10.1016/j.immuni.2012.11.018

Ye J, Zhu B, Fu ZF et al (2013) Immune evasion strategies of flaviviruses. Vaccine 31:461–471. doi:10.1016/j.vaccine.2012.11.015

Zhang P, Snyder S, Feng P et al (2006) Role of N-acetylglucosamine within core lipopolysaccharide of several species of gram-negative bacteria in targeting the DC-SIGN (CD209). J Immunol 177:4002–4011. doi:10.4049/jimmunol.177.6.4002

Zhao LJ, Wang W, Ren H, Qi ZT (2013) ERK signaling is triggered by hepatitis C virus E2 protein through DC-SIGN. Cell Stress Chaperones 18:495–501. doi:10.1007/s12192-013-0405-3