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# Mannose–fucose recognition by DC-SIGN

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## SUMMARY

Dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) is a C-type lectin receptor that recognizes *N*-linked high-mannose oligosaccharides and branched fucosylated structures. It is primarily expressed by dendritic cells and mediates the capture, destruction and presentation of microbial pathogens to induce successful immune responses. Furthermore, DC-SIGN is involved in the priming of T-cell responses and facilitates dendritic cell homeostasis by controlling extravasation into peripheral tissues. However, an increasing amount of evidence suggests that pathogens also exploit DC-SIGN to subvert host immune responses. Herein, we discuss the current state of knowledge of DC-SIGN-carbohydrate interactions and investigate how these interactions influence dendritic cell functioning. First, an overview of the structure of DC-SIGN is provided and its expression pattern among immune cells is discussed. After this, the molecular aspects that underlie the selectivity of DC-SIGN for mannose- and fucose-containing carbohydrates are detailed. Finally, the role of DC-SIGN in dendritic cell biology is discussed and how certain bacterial pathogens exploit DC-SIGN to escape immune surveillance.

*Keywords:* C-Type lectin; Dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN); High-mannose; Lewis antigens; Carbohydrate recognition; Dendritic cell; Immune suppression; Host-pathogen interaction; Pathogenesis; Virulence

## 1. INTRODUCTION

The capability of the innate immune system quickly to detect and respond to invading pathogens is essential for controlling infection. Pattern-recognition receptors (PRRs) have evolved to recognize a wide variety of pathogen-associated molecular patterns (PAMPs) and trigger antimicrobial responses in immune cells. Recognition of PAMPs by PRRs is an important determinant for the overall quality and effectiveness of immune responses by mediating not only direct effector functions, such as phagocytosis and degranulation, but also by transmitting signals that regulate the expression of genes important for both innate and adaptive immune responses. One of the best studied classes of PRRs are the Toll-like

receptors (TLRs). This receptor family recognizes a diverse range of microbial compounds and, upon ligation, trigger robust innate immune responses by inducing proinflammatory cytokine and chemokine production and by upregulating co-stimulatory molecule expression (Akira and Takeda, 2004). Although TLRs are an important warning system that alerts host immune cells to the presence of invading microbes, it remains unclear whether they also facilitate antigen uptake and it seems that this process is mediated by other types of PRRs, such as scavenger receptors (Murphy *et al.*, 2005) and C-type lectin receptors (CLRs) (Cambi *et al.*, 2005; McGreal *et al.*, 2005). The C-type lectin receptors encompass a large family of proteins that are best known for their ability to detect and capture microbe-derived materials. Classical CLRs contain calcium- ( $\text{Ca}^{2+}$ -) dependent carbohydrate recognition domains (CRDs) which are involved in the recognition of carbohydrate structures on both self and non-self ligands. The C-type lectin CRDs are part of a large family of protein domains called C-type lectin domains (CTLDs) which are characterized by a common protein-fold consisting of two anti-parallel  $\beta$ -strands and two  $\alpha$ -helices (Weis *et al.*, 1998; Drickamer, 1999). Although the name C-type lectin refers to a  $\text{Ca}^{2+}$ -dependent carbohydrate-binding protein, it is now clear that this name is a misnomer and that many C-type lectins are calcium-independent and do not bind carbohydrates but rather recognize proteins or lipids. Therefore, the term C-type lectin does not refer to a protein family which shares functional similarities but rather defines a class of proteins that all contain one or more CTLDs.

The C-type lectin receptors exhibit important functions during primary immune responses (Robinson *et al.*, 2006). Besides their role in pathogen detection and uptake via PAMPs, they also mediate cell-cell interactions by the recognition of endogenous ligands (Cambi and Figdor, 2003). Furthermore, they are involved in the induction of immune tolerance and play a role in endogenous glycoprotein homeostasis (Cambi

and Figdor, 2003). In addition, C-type lectins have been shown to influence TLR-mediated responses (Mukhopadhyay *et al.*, 2004; Cambi *et al.*, 2005). Moreover, C-type lectins can be produced as transmembrane proteins or as soluble, secreted proteins. Examples of soluble C-type lectins are the members of the collectins family, such as lung surfactant proteins A and D (Pastva *et al.*, 2007), and the plasma-localized mannose-binding lectin (MBL) (Takahashi *et al.*, 2006) (see Chapter 35). Transmembrane C-type lectins can be divided into two groups based upon the orientation of their amino (N)-terminus. Myeloid cells predominantly express type-II C-type lectins. These lectins all contain a single CRD and have been shown to bind a wide variety of carbohydrate ligands. Examples of myeloid type-II C-type lectins are the dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and its close relative DC-SIGNR (L-SIGN), the related murine receptor family termed SIGN-related (SIGNR) 1-4, Langerin, blood DC antigen 2 (BDCA-2), C-type lectin receptors 1 and 2 (CLEC-1 and CLEC-2, respectively), macrophage galactose-type lectin (MGL), DC immunoreceptor (DCIR) and Dectin-1 and -2 (Cambi *et al.*, 2005; McGreal *et al.*, 2005; Robinson *et al.*, 2006). Myeloid type-I C-type lectins contain multiple CRDs. Examples of these lectins are the macrophage mannose receptor (MR), the phospholipase  $A_2$  receptor, DEC-205 and Endo-180 (Apostolopoulos and McKenzie, 2001; East and Isacke, 2002). Investigations on the carbohydrate-binding specificities of  $\text{Ca}^{2+}$ -dependent CRDs have shown that they fall within two broad categories: those recognizing D-mannose- (Man-) like structures and those recognizing D-galactose- (Gal-) like structures. This subdivision is supported at the molecular level by studies demonstrating that Man- and Gal-binding CRDs contain characteristic amino acid triplets: EPN (in one-letter amino acid code) for Man-binding CRDs and QDP (in one-letter amino acid code) for Gal-binding CRDs (Drickamer, 1992). In addition, some CLRs

(e.g. MR) can recognize sulfated carbohydrates independent of their CRD. This interaction is mediated by a cysteine-rich domain which is present in these proteins (Fiete *et al.*, 1998).

Indeed, DC-SIGN is a dendritic cell (DC)-specific type-II C-type lectin which was originally identified as a receptor that specifically interacts with the intercellular adhesion molecule (ICAM)-3 on T-cells, thereby mediating DC-T-cell interactions (Geijtenbeek *et al.*, 2000b). Later on, DC-SIGN was also shown to be involved in DC migration by interacting with ICAM-2 on vascular endothelial cells (Geijtenbeek *et al.*, 2000a). Ever since its discovery, DC-SIGN received major scientific interest. This interest grew further when it became clear that DC-SIGN has a dual function in that it also recognizes a wide variety of pathogens (Table 34.1). Investigations on its carbohydrate-specificity using glyco-arrays have shown that DC-SIGN preferentially binds Man- and L-fucose- (Fuc-) containing structures. In this chapter, we summarize the current state of knowledge on DC-SIGN-carbohydrate interactions. First, the structure of DC-SIGN and its expression patterns among various immune cells is discussed. Then the molecular aspects that underlie the selectivity of DC-SIGN for Man- and Fuc-containing carbohydrates are detailed. Finally, the role of DC-SIGN in DC biology and how certain pathogens exploit DC-SIGN to escape immune surveillance are reviewed.

## 2. DC-SIGN STRUCTURE AND EXPRESSION

### 2.1. Structure of DC-SIGN

Human DC-SIGN (CD209) is a 404 amino acid protein encoded in seven exons on chromosome 19p13. Upstream, in the reverse orientation, and downstream of DC-SIGN, two DC-SIGN homologues are encoded: DC-SIGNR

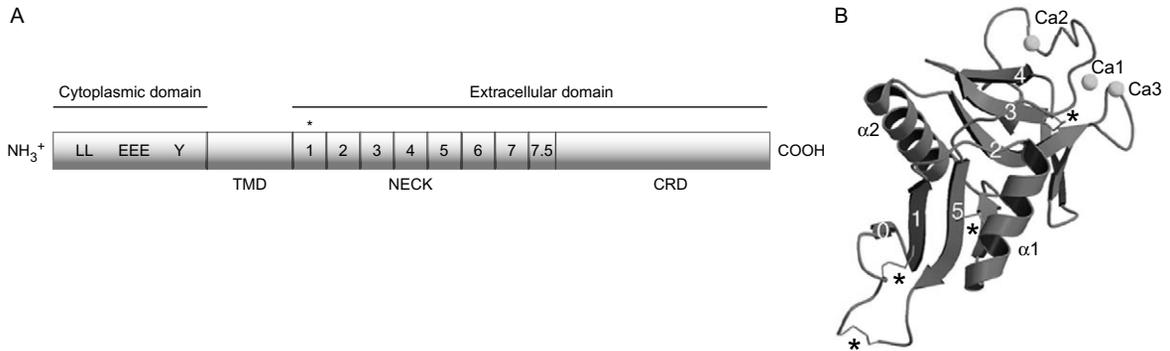
which shows 77% amino acid sequence identity to DC-SIGN and LSEctin which has 31% identity to DC-SIGN, respectively (Soilleux *et al.*, 2000; Liu *et al.*, 2004b). Further downstream lies the gene encoding the low affinity IgE receptor (CD23), a C-type lectin with a gene organization similar to DC-SIGN (Soilleux *et al.*, 2000). Structurally, DC-SIGN itself is a prototype type II transmembrane protein consisting of a carboxy (C)-terminal CRD followed by a neck domain fused to a transmembrane region and ending with a N-terminal cytoplasmic tail, which harbours internalization and recycling motifs, such as a di-leucine (LL) and a tri-acidic (EEE) motif, and an incomplete immunoreceptor tyrosine-based activation motif (ITAM) (van Kooyk and Geijtenbeek, 2003) (Figure 34.1A). Crystal structures of the CRDs of DC-SIGN and DC-SIGNR revealed that both CRDs exhibit a typical long-form C-type lectin fold (Feinberg *et al.*, 2001) (Figure 34.1B). The structure harbours three  $\text{Ca}^{2+}$  ions, of which one, the principal  $\text{Ca}^{2+}$  (see Figure 34.1B;  $\text{Ca}^{2+}$  no. 2), is common to all  $\text{Ca}^{2+}$ -dependent C-type lectins and dictates the recognition of specific carbohydrate structures. The principal  $\text{Ca}^{2+}$  interacts with four amino acids; Glu347, Asn349, Glu354 and Asn365 in DC-SIGN (where Glu, glutamic acid; Asn, asparagine) and Glu359, Asn361, Glu366 and Asn377 in DC-SIGNR and mutations in these amino acids lead to loss of ligand interaction (Geijtenbeek *et al.*, 2002b). The DC-SIGN neck domain is composed of one incomplete and seven complete 23-amino acid tandem repeats which are encoded in a single exon. The number of neck repeats is highly conserved, although polymorphisms have been reported (Liu *et al.*, 2004a; Barreiro *et al.*, 2007; Ben-Ali *et al.*, 2007; Rathore *et al.*, 2008). In contrast, the number of neck repeats in DC-SIGNR is much more variable and varies between four and nine (Bashirova *et al.*, 2001; Feinberg *et al.*, 2005). Cross-linking and analytical ultracentrifugation experiments have shown that purified recombinant DC-SIGN and DC-SIGNR CRDs

TABLE 34.1 Pathogens recognized by DC-SIGN

Pathogen	Ligand <sup>b</sup>	Carbohydrate epitope
<b>Viruses<sup>a</sup></b>		
HIV-1	gp120	High-mannose
HIV-2	gp120	Unknown
SIV	gp120	Unknown
FIV	gp95	Unknown
Ebola virus	Glycoprotein	High-mannose
Marburg virus	Glycoprotein	Unknown
Cytomegalovirus	Glycoprotein B	Unknown
Hepatitis C virus	E1/E2 glycoproteins	Unknown
Dengue virus	Glycoprotein E	N-linked glycans
Alpha viruses	Unknown	Unknown
SARS corona virus	S protein	N-linked glycans
West Nile virus	Glycoprotein E	N-linked glycans
Human herpes virus 8	Unknown	Unknown
Measles virus	Glycoproteins F and H	Unknown
<b>Bacteria</b>		
<i>Helicobacter pylori</i>	LPS	Lewis antigens
<i>Mycobacterium</i> spp.	ManLAM, PIMs, LM, AM, 19- and 45-kDa antigens	Di- and tri-mannose
<i>Lactobacillus</i> spp.	S-layer protein A	Unknown
<i>Escherichia coli</i>	LPS	N-acetyl-D-glucosamine
<i>Salmonella enterica</i>	LPS	N-acetyl-D-glucosamine
<i>Haemophilus ducreyi</i>	LPS	N-acetyl-D-glucosamine
<i>Neisseria</i> spp.	IgtB LPS	N-acetyl-D-glucosamine
<i>Streptococcus pneumoniae</i>	Capsular polysaccharides and unknown	Unknown
<i>Yersinia pestis</i>	LPS	Unknown
<b>Fungi</b>		
<i>Candida albicans</i>	N-linked mannan	Mannose
<i>Aspergillus fumigatus</i>	Unknown	Unknown
<i>Chrysosporium tropicum</i>	Unknown	Unknown
<b>Parasites</b>		
<i>Leishmania</i> spp.	Unknown	Unknown
<i>Schistosoma mansoni</i>	SEAs and glycolipids	Lewis <sup>x</sup> and pseudo-Lewis <sup>y</sup>
<i>Toxocara canis</i>	Excretory/secretory products	Unknown

<sup>a</sup>Viral abbreviations: HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; FIV, feline immunodeficiency virus; SARS, severe acute respiratory syndrome.

<sup>b</sup>Compound abbreviations: AM, arabinomannan; LM, lipomannan; LPS, lipopolysaccharide; ManLAM, mannose-capped lipoarabinomannan; PIMs, phosphatidylinositol-mannosides; SEAs, soluble egg antigens.



**FIGURE 34.1** Structure of DC-SIGN. (A) Schematic representation of DC-SIGN. DC-SIGN is a prototype type II transmembrane protein consisting of a carboxy- (COOH-) terminal carbohydrate recognition domain (CRD), followed by a neck domain consisting of one incomplete and seven complete tandem repeats (numbered 1–7.5) fused to a transmembrane domain (TMD) and ending with an amino- (NH<sub>3</sub><sup>+</sup>-) terminal cytoplasmic tail. The latter harbours internalization and recycling motifs, such as a di-leucine (LL) and a tri-acidic (EEE) motif, and an incomplete immunoreceptor tyrosine- (Y-) based activation motif (ITAM). An asterisk indicates the N-glycosylation site situated in neck repeat 1. (B) Ribbon diagram of the CRD of DC-SIGN. Large spheres represent the three Ca<sup>2+</sup> ions (ions are numbered Ca1–Ca3). Disulfide bonds are indicated by an asterisk. The CRD of DC-SIGNR resembles that of DC-SIGN, except for the disulfide bond connecting the carboxy- and amino-termini, which cannot be seen in DC-SIGNR. Figure 34.1B was adapted, with permission, from Feinberg *et al.* (2001) Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* 294, 2163–2166. © AAAS.

exist as monomers, whereas the complete extracellular domains form tetramers (Mitchell *et al.*, 2001; Feinberg *et al.*, 2005). Analysis by circular dichroism spectroscopy indicated that the neck region has a high  $\alpha$ -helical content and forms a tetrameric coiled-coil structure that projects the CRDs away from the cell surface (Mitchell *et al.*, 2001). Furthermore, it was demonstrated that tetramerization enhances the affinity for multivalent ligands (Mitchell *et al.*, 2001). A disadvantage of using recombinant proteins is that they may exhibit differential glycosylation and therefore behave differently from their natural equivalents. In a recent study, which investigated the multimerization and ligand-binding activities of various DC-SIGN splice forms, it was shown that N-glycosylation of Asn80 in repeat 1 negatively influences multimer formation (Serrano-Gomez *et al.*, 2008). Furthermore, it was demonstrated that the presence of the two most N-terminal repeats is sufficient for multimerization, that the specific order of the

neck repeats is important for functionality and that the CRD contributes to tetramer stabilization via cysteine-mediated interactions (Serrano-Gomez *et al.*, 2008). Interestingly, the authors also found that, although the capacity to multimerize correlates with the ability to bind soluble carbohydrates, it does not correlate with the capability to interact with certain pathogens, such as *Candida albicans* and *Leishmania infantum* (Serrano-Gomez *et al.*, 2008). A possible explanation is that the large amount of DC-SIGN ligands on the surface of interacting pathogens compensates for the distinct affinities and multimerization abilities of DC-SIGN isoforms.

## 2.2. Expression of DC-SIGN

DC-SIGN is expressed by DCs both *in vitro* and *in vivo* (Bleijis *et al.*, 2001). Monocyte- and CD34<sup>+</sup>-derived DCs abundantly express DC-SIGN (Geijtenbeek *et al.*, 2000b). It is not

expressed by monocytes, monocytic cell lines, activated monocytes, granulocytes, CD34<sup>+</sup> bone marrow cells or T- and B-lymphocytes (Geijtenbeek *et al.*, 2000b). *In vivo*, DC-SIGN is expressed by immature DCs (iDCs) in lymphoid and fibrous connective tissues and in mucosae, but not by epidermal CD1a<sup>+</sup> Langerhans cells (Geijtenbeek *et al.*, 2000a,c; Soilleux and Coleman, 2001; Soilleux *et al.*, 2002). The percentage of DC-SIGN-positive blood cells is very low and only certain subsets of blood DCs seem to express DC-SIGN (Engering *et al.*, 2002b; Soilleux *et al.*, 2002). Although DC-SIGN was thought to be DC-specific, its expression has now been demonstrated on specific macrophage subsets, such as decidual and alveolar macrophages and on Hofbauer cells in the placenta (Geijtenbeek *et al.*, 2001; Lee *et al.*, 2001; Soilleux *et al.*, 2001, 2002). Interestingly, studies in fetal tissues have demonstrated that while fetal iDCs normally express DC-SIGN, alveolar macrophages do not (Soilleux *et al.*, 2002). This indicates that DC-SIGN expression in this type of macrophage may be dependent on antigenic stimulation. Consistent with this hypothesis is the finding that interleukin- (IL-) 13 treatment of monocyte-derived macrophages induces DC-SIGN expression (Soilleux *et al.*, 2002). This observation, together with the ability of IL-4 to induce monocytic DC-SIGN expression (Relloso *et al.*, 2002) and the genetic linkage between DC-SIGN and CD23, which is part of the T-helper-2 cell (Th2) axis of immunity, suggests that DC-SIGN plays a role in Th2-type immune responses (Soilleux *et al.*, 2000; Park *et al.*, 2001; Soilleux, 2003).

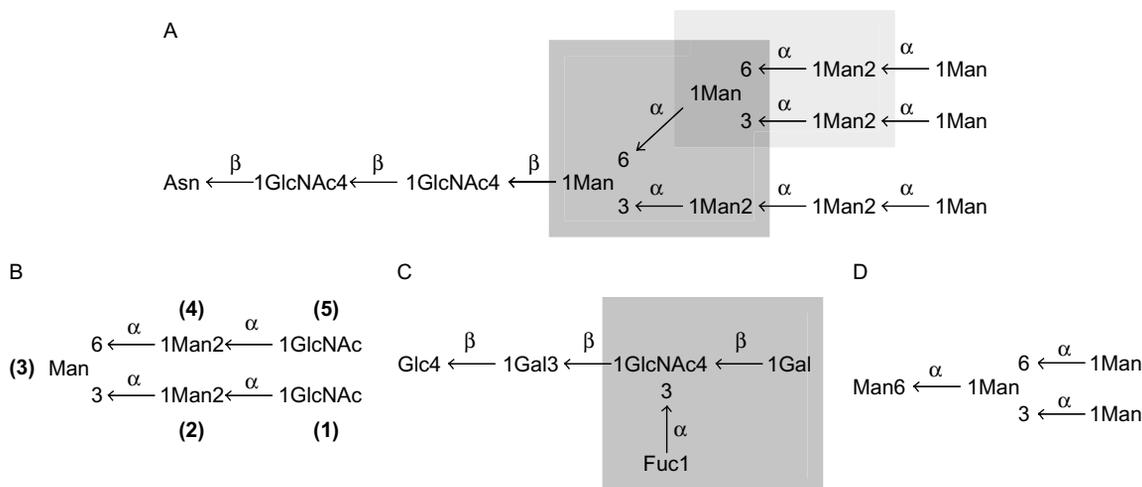
### 3. SELECTIVE RECOGNITION OF MAN- AND FUC-CONTAINING GLYCANS BY DC-SIGN

Glycan array screening using recombinant DC-SIGN fragments or DC-SIGN extracellular

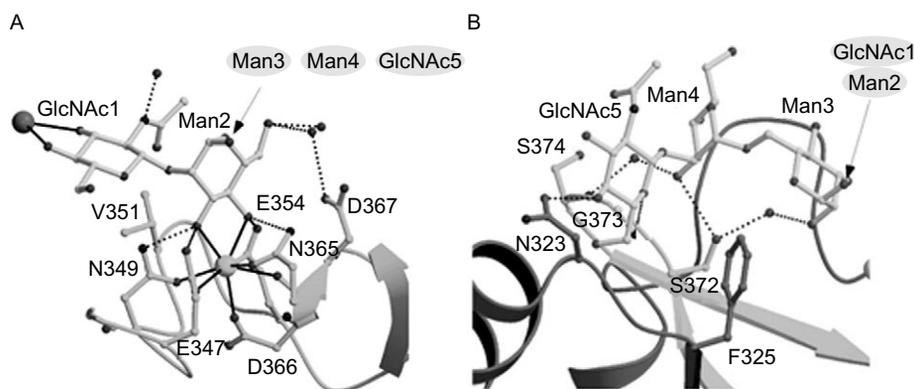
domains fused to an Fc domain (DC-SIGN-Fc) have demonstrated that DC-SIGN recognizes two classes of carbohydrates: N-linked high-Man oligosaccharides and branched fucosylated structures such as the Lewis (Le) blood group antigens, Le<sup>a</sup>, Le<sup>b</sup>, Le<sup>x</sup>, and Le<sup>y</sup> (Feinberg *et al.*, 2001, 2007; Appelmelk *et al.*, 2003; Guo *et al.*, 2004; van Liempt *et al.*, 2006). Also, DC-SIGNR, which is 77% homologous to DC-SIGN, displays a similar specificity, except for the Le<sup>x</sup> epitope which is only recognized by DC-SIGN (Appelmelk *et al.*, 2003; van Kooyk *et al.*, 2003; van Die *et al.*, 2003; Guo *et al.*, 2004; van Liempt *et al.*, 2004). The hallmark of carbohydrate binding to C-type lectins is the primary interaction between the principal Ca<sup>2+</sup> and the vicinal hydroxyl groups of a pyranose ring. Further substrate specificity comes from secondary interactions between the carbohydrate and CRD-specific amino acids.

#### 3.1. DC-SIGN binding to high-Man oligosaccharides

Solid-phase competition and binding assays have shown that DC-SIGN and DC-SIGNR interact with N-linked high-Man structures (Feinberg *et al.*, 2001, 2007; Guo *et al.*, 2004; van Liempt *et al.*, 2006). Both proteins show the highest apparent affinity towards structures containing nine mannosyl residues, i.e. Man<sub>9</sub>GlcNAc<sub>2</sub> (where GlcNAc, N-acetyl-D-glucosamine) (Figure 34.2(A)). Co-crystallization experiments have shown that the interaction between DC-SIGN/DC-SIGNR and high-Man structures is mediated by vicinal, equatorial 3- and 4-OH groups of internal mannosyl residues (Feinberg *et al.*, 2001, 2007; Guo *et al.*, 2004) (Figure 34.3A). The preference for internal residues is unusual since most Man-binding lectins (e.g. MR and MBL) recognize terminal residues (Weis *et al.*, 1992; Hitchen *et al.*, 1998). In the first crystal structure that was published, DC-SIGN and DC-SIGNR were co-crystallized with the pentasaccharide



**FIGURE 34.2** Schematic representations of mannose- (Man-) containing structures that interact with DC-SIGN and DC-SIGNR. (A) the *N*-linked high-mannose (Man) structure  $\text{Man}_9\text{GlcNAc}_2$ ; the inner branched trimannose structure  $\alpha\text{-Man-(1}\rightarrow\text{3)-}[\alpha\text{-Man-(1}\rightarrow\text{6)-}]\text{Man}$  is indicated by the dark grey box, whereas the outer tri-Man structure is indicated by the light grey box; (B) the pentasaccharide  $\text{Man}_3\text{GlcNAc}_2$  that has been co-crystallized with DC-SIGN and DC-SIGNR (residues are numbered as mentioned in the text); (C) the lacto-*N*-fucopentaose III pentasaccharide; and (D) the  $\text{Man}_4$  oligosaccharide structures that were co-crystallized with DC-SIGN. For lacto-*N*-fucopentaose III, the  $\text{Le}^x$  structure is highlighted by the grey box. Abbreviations: Asn, asparagine; Fuc, L-fucose; Man, D-mannose; Gal, D-galactose; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine. In part adapted, with permission, from Feinberg *et al.* (2001) Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* 294, 2163–2166. © AAAS.



**FIGURE 34.3** Co-crystallization of  $\text{Man}_3\text{GlcNAc}_2$  with DC-SIGN and DC-SIGNR. (A) Interaction of the  $\alpha\text{-}(1\rightarrow\text{3})$ -linked branch of  $\text{Man}_3\text{GlcNAc}_2$  with the carbohydrate recognition domain (CRD) of DC-SIGN. For clarity, some of the sugar residues are shown schematically. Large spheres represent  $\text{Ca}^{2+}$  ions. The  $\text{Ca}^{2+}$  coordination bonds are shown as solid black lines; hydrogen bonds as dashed lines. Important residues are numbered. Of note, the terminal GlcNAc1 interacts with the principal  $\text{Ca}^{2+}$  ion of another DC-SIGN CRD (Note the second CRD is not shown). (B) Interaction of the  $\alpha\text{-}(1\rightarrow\text{6})$ -linked branch of  $\text{Man}_3\text{GlcNAc}_2$  with the CRD of DC-SIGNR. Hydrogen bonds are shown as dashed lines and important residues are numbered. Abbreviations: GlcNAc, *N*-acetyl-D-glucosamine; Man, D-mannose. Derived, with permission, from Feinberg *et al.* (2001) Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* 294, 2163–2166. © AAAS.



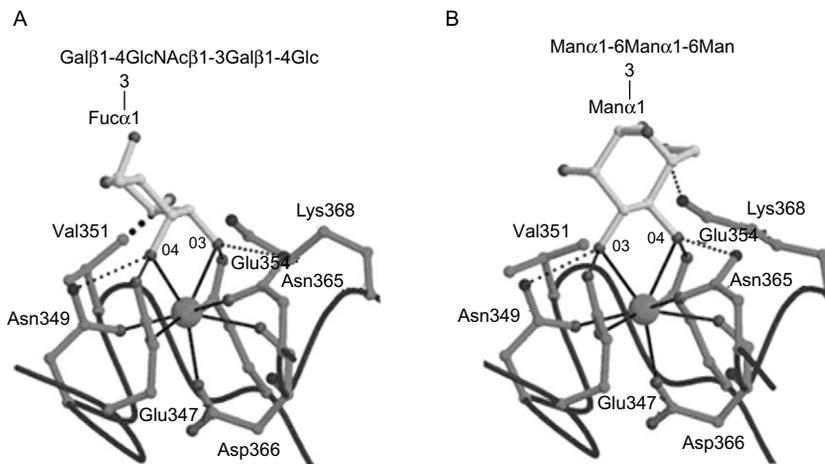
Interestingly, it was recently shown that DC-SIGN is able to bind an  $\alpha$ -Man-(1 $\rightarrow$ 2)-Man disaccharide (either by itself or as part of a Man<sub>6</sub> structure) independently of the  $\alpha$ -Man-(1 $\rightarrow$ 3)-[ $\alpha$ -Man-(1 $\rightarrow$ 6)-]Man trisaccharide (Feinberg *et al.*, 2007). This interaction encompassed not only the principal Ca<sup>2+</sup> but also included a valine residue at position 351. This result suggests that the observed affinity enhancement towards oligosaccharides that besides a tri-Man core also contain terminal  $\alpha$ -Man-(1 $\rightarrow$ 2)-Man groups (e.g. Man<sub>6</sub>GlcNAc<sub>2</sub>) is due to multiple binding modes to the CRD, which provide both additional contacts (mediated by Val351) and a statistical (entropic) enhancement of binding (Feinberg *et al.*, 2007).

### 3.2. DC-SIGN binding to branched Fuc structures

The first observations that DC-SIGN binds branched fucosylated structures were made in studies examining the interaction between DC-SIGN and surface glycans of human pathogens (Appelmelk *et al.*, 2003; van Die *et al.*, 2003). In one of these studies, it was observed that the interaction between *Schistosoma mansoni* soluble egg antigens (SEAs) and DC-SIGN could be inhibited by antibodies recognizing Le<sup>x</sup>,  $\beta$ -Gal-(1 $\rightarrow$ 4)-[ $\alpha$ -Fuc-(1 $\rightarrow$ 3)-]GlcNAc and N-acetyl-D-galactosamine- (GalNAc-) containing LDNF,  $\beta$ -GalNAc-(1 $\rightarrow$ 4)-[ $\alpha$ -Fuc-(1 $\rightarrow$ 3)-]GlcNAc, epitopes (both present on SEAs), thereby suggesting that these structures bind to DC-SIGN (van Die *et al.*, 2003). This was supported by the observation that DC-SIGN-Fc specifically bound to polyvalent neo-glycoconjugates harbouring the Le<sup>x</sup> epitope. In addition, it was shown that DC-SIGN binds to related Le epitopes, such as: Le<sup>a</sup>,  $\beta$ -Gal-(1 $\rightarrow$ 3)-[Fuc- $\alpha$ -(1 $\rightarrow$ 4)-]GlcNAc; Le<sup>b</sup>,  $\alpha$ -Fuc-(1 $\rightarrow$ 2)- $\beta$ -Gal-(1 $\rightarrow$ 3)-[Fuc- $\alpha$ -(1 $\rightarrow$ 4)-]GlcNAc; and Le<sup>y</sup>,  $\alpha$ -Fuc-(1 $\rightarrow$ 2)- $\beta$ -Gal-(1 $\rightarrow$ 4)- [  $\alpha$ -Fuc-(1 $\rightarrow$ 3)-]GlcNAc but not to sulfo- or sialyl-Le<sup>x</sup> (Appelmelk *et al.*, 2003).

Similar to DC-SIGN, DC-SIGNR can recognize Le<sup>a</sup>, Le<sup>b</sup> and Le<sup>y</sup> epitopes (van Liempt *et al.*, 2004). However, DC-SIGNR does not bind to the Le<sup>x</sup> epitope (Guo *et al.*, 2004; van Liempt *et al.*, 2004). Insight into the molecular mechanism underlying this differential recognition was provided when the CRD of DC-SIGN was co-crystallized with the Le<sup>x</sup>-containing pentasaccharide lacto-N-fucopentaose III (Guo *et al.*, 2004) (see Figures 34.2C and 34.5A). The structure revealed that the interaction between Le<sup>x</sup> and the principal Ca<sup>2+</sup> was mediated by the  $\alpha$ -(1 $\rightarrow$ 3)-linked Fuc residue. Because in Fuc the 3-OH group is in equatorial and the 4-OH group is in axial conformation, the Fuc ring was tipped compared to the mannosyl residue (for Man both the 3- and 4-OH groups are in equatorial conformation) in a structure of DC-SIGN co-crystallized with a Man<sub>4</sub> oligosaccharide (Guo *et al.*, 2004) (see Figures 34.2D and 34.5B). As a consequence of this orientation, the Fuc ring is close to Val351, which forms tight van der Waals contacts with its 2-OH group. In addition, the terminal Gal residue binds to a secondary binding site encompassing Glu358, Asp367, Lys368, Leu371 and Lys373 (where Glu, glutamic acid; Asp, aspartic acid; Lys, lysine; Leu, leucine) (Guo *et al.*, 2004).

Interestingly, the Val351 residue is substituted for a serine residue (Ser363) in DC-SIGNR, eliminating the van der Waals contact with the 2-OH group. This feature, together with a subtle difference in ligand orientation due to differences in amino acid sequence can explain the inability of DC-SIGNR to bind Le<sup>x</sup>. This is supported by the observation that substituting Ser363 in DC-SIGNR for a valine residue enables it to bind to Le<sup>x</sup> epitopes (Guo *et al.*, 2004; van Liempt *et al.*, 2004). The reason why DC-SIGNR does not bind Le<sup>x</sup> but is able to interact with closely related structures such as Le<sup>a</sup> and Le<sup>y</sup> remains unclear and probably involves differences in stericity and/or bulkiness of the corresponding structures. Further co-crystallization experiments of DC-SIGN and DC-SIGNR with other Le antigens



**FIGURE 34.5** Co-crystallization of lacto-*N*-fucopentaose III and a  $\text{Man}_4$  oligosaccharide with DC-SIGN. Close-up of (A) the  $\alpha$ -(1 $\rightarrow$ 3)-linked fucose residue of lacto-*N*-fucopentaose III or (B) the terminal, non-reducing  $\alpha$ -(1 $\rightarrow$ 3)-linked mannose residue of the  $\text{Man}_4$  oligosaccharide in the primary binding site of DC-SIGN. The large spheres represent the principal  $\text{Ca}^{2+}$  ions. The  $\text{Ca}^{2+}$  coordination bonds are shown as solid black lines, hydrogen bonds as thin dashed lines and van der Waals contacts as thick dashed lines. Important residues are numbered. Abbreviations: Asn, asparagines; Asp, aspartic acid; Fuc, L-fucose; Lys, lysine; Man, D-mannose; Gal, D-galactose; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; Glu, glutamic acid; Val, valine. Reproduced, with permission, from Guo *et al.* (2004) Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. *Nat. Struct. Mol. Biol.* 11, 591–598.

are needed to obtain insight into the mechanism underlying this differential recognition.

#### 4. IN VIVO FUNCTION AND ROLE IN DENDRITIC CELL BIOLOGY OF DC-SIGN

Dendritic cells are professional antigen presenting cells crucial for mounting successful immune responses against invading pathogens and for maintaining immune tolerance towards endogenous ligands. The iDCs originate from the bone marrow where they develop from both myeloid- and lymphoid-committed progenitors (Wu and Liu, 2007). After entering the blood stream, iDCs extravasate into peripheral tissues where they continuously sample their environment by capturing antigens and subsequently presenting them on major histocompatibility

(MHC) complexes. Upon pathogen encounter, iDCs receive activation signals that trigger their maturation and stimulate migration into secondary lymphoid organs where they interact with naïve T-cells and initiate an adaptive immune response. Both DC maturation and migration are tightly controlled processes dictated by a variety of cytokines, chemokines and adhesion molecules. Adhesion molecules are not only important for facilitating cellular interactions involved in DC migration, i.e. DC-endothelial cell interactions, but also play a role in establishing DC-T-cell contacts that enable MHC scanning and T-cell receptor activation.

##### 4.1. DC-SIGN facilitates DC migration from the blood into tissues

Migration of iDCs into surrounding tissues is an important process that is pivotal for the

successful detection and eradication of invading pathogens. In recent years, the molecular mechanisms underlying DC migration have become increasingly clear and several molecules are now known to be involved, including various C-type lectins such as the MR, the selectins and DC-SIGN (Irjala *et al.*, 2001; Geijtenbeek *et al.*, 2002a, 2004). Indeed, DC-SIGN was shown to mediate the rolling and adhesion of iDCs on vascular endothelial cells by interacting with ICAM-2 (Geijtenbeek *et al.*, 2000a). Furthermore, the DC-SIGN-ICAM-2 interaction was shown to facilitate the transmigration of iDCs across both resting and activated endothelia (Geijtenbeek *et al.*, 2000a). Since ICAM-2 expression is constitutive and does not depend on endothelial activation (Nortamo *et al.*, 1991), DC-SIGN-ICAM-2 interactions probably play a central role in homeostatic extravasation of iDCs into surrounding tissues. Due to its heavy glycosylation, it has long been unclear what particular ICAM-2 structure is recognized by DC-SIGN. Although high-Man structures were initially suspected (Jimenez *et al.*, 2005), it was recently demonstrated that the interaction is primarily dependent on Le<sup>x</sup> epitopes present on ICAM-2 (Garcia-Vallejo *et al.*, 2008).

#### 4.2. DC-SIGN mediates DC-T-cell interactions

After receiving activation signals, iDCs become mature and migrate to secondary lymphoid organs where they interact with T-cells and stimulate an adaptive immune response. The initial DC-T-cell contact is transient and allows for the rapid scanning of MHC complexes by T-cells. Studies aimed at identifying molecules important in this process suggested the involvement of ICAM-3 (Hauss *et al.*, 1995; Starling *et al.*, 1995). Initially, it was thought that T-cell ICAM-3 interacted with leukocyte function antigen-1 (LFA-1) on DCs (Hauss *et al.*, 1995), however, later on, it was shown that the

affinity of this interaction is low and that the actual DC counter-receptor is DC-SIGN (Geijtenbeek *et al.*, 2000b). It was demonstrated that DC-SIGN binds ICAM-3 with high affinity and forms the major ICAM-3 receptor on DCs. Monoclonal antibodies blocking DC-SIGN inhibited DC-T-cell clustering and prevented DC-dependent proliferation of resting T-cells. Furthermore, it was shown that the DC-SIGN-ICAM-3 interaction is transient, which allows for the rapid screening of MHC-peptide complexes (Geijtenbeek *et al.*, 2000b). Although these data seem strong and the interaction between DC-SIGN and ICAM-3 has been confirmed independently (Geijtenbeek *et al.*, 2002b; Su *et al.*, 2004; Jimenez *et al.*, 2005), a potential problem may be that these studies were performed using recombinant proteins. It is well known that protein glycosylation is not only dependent on the proteins themselves but is also influenced by the nature of the cell line in which they are expressed and the cell culture conditions that are used (Brooks, 2006; Devasahayam, 2007). The possibility that the utilization of recombinant proteins may have led to misinterpretation was raised in a recent paper in which the interactions between DC-SIGN-Fc and ICAM-3 molecules directly purified from blood leukocytes were investigated (Bogoevska *et al.*, 2007). Unexpectedly, this study found that DC-SIGN-Fc could only bind to ICAM-3 molecules that were derived from granulocytes and not to ICAM-3 that was isolated from monocytes, B-cells and, most importantly, T-cells. Moreover, it was found that the interaction was dependent on Le<sup>x</sup> and could be abolished by pretreating ICAM-3 with fucosidase III (Bogoevska *et al.*, 2007). The presence of Le<sup>x</sup> epitopes on ICAM-3 was confirmed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and an antibody direct against Le<sup>x</sup>, which only recognized granulocyte ICAM-3 and not the ICAM-3 of other cell types. Surprisingly, these observations do not support the earlier finding that ICAM-3 plays

an important role in DC-T-cell interactions (Geijtenbeek *et al.*, 2000b). Furthermore, they contradict an earlier study in which it was shown that native, neutrophil-derived ICAM-3 binds DC-SIGN-Fc with poor affinity (van Gisbergen *et al.*, 2005a). Possible explanations for these conflicting results may be:

- (i) the genetic variability between different donors;
- (ii) differences in ICAM-3 preparations due to alternative isolation procedures (immunoprecipitation versus affinity chromatography); and
- (iii) structural differences between the DC-SIGN-Fc constructs that were used.

Whereas Bogoevska *et al.* (2007) used an N-terminal DC-SIGN-Fc fusion protein produced in human embryonic kidney 293 cells, van Gisbergen *et al.* (2005a) used a C-terminal DC-SIGN-Fc fusion protein prepared from Chinese hamster ovary K1 cells. That the origin of recombinant soluble lectin can indeed determine specificity was recently shown for the interaction of the NK-cell lectin Nkp30 with heparan sulfate (Hershkovitz *et al.*, 2008). Furthermore, van Gisbergen *et al.* (2005a) did not include a control for the amount of ICAM-3 that was present on their blots. Nevertheless, the observation that the interaction of T-cells with iDCs can be blocked by monoclonal antibodies against DC-SIGN (Geijtenbeek *et al.*, 2000b) suggests that DC-SIGN plays an important role in early DC-T-cell contacts. The question whether these interactions are indeed dependent on ICAM-3 or, as has now been suggested, are mediated by different molecules remains open and awaits further investigation.

### 4.3. Role of DC-SIGN in DC-neutrophil interactions

Neutrophils are phagocytic cells that play an important role in innate immunity by engulfing

and killing extracellular pathogens. Upon infection, neutrophils infiltrate inflamed tissues where they kill pathogens through phagocytosis and the release of antimicrobial compounds. Although primarily beneficial, neutrophil-derived oxidants, proteinases, cationic peptides and reactive oxygen species can damage surrounding tissues (Moraes *et al.*, 2006). For this reason, neutrophil turnover is a tightly controlled process which is dependent on a fine balance between pre- and anti-apoptotic signals (Walker *et al.*, 2005). In addition, neutrophils play a role in adaptive immune responses not only by recruiting additional immune cells to the site of infection but also by directly influencing their activity. More recently, it was shown that neutrophils can directly interact with iDCs (van Gisbergen *et al.*, 2005a). It was demonstrated that this interaction is dependent on DC-SIGN (van Gisbergen *et al.*, 2005a). Up until now, three potential neutrophil DC-SIGN-ligands have been identified. Besides ICAM-3, also Mac-1 (CD11b/CD18) and the carcinoembryonic antigen-related cellular adhesion molecule (CEACAM) 1 were shown to bind DC-SIGN (van Gisbergen *et al.*, 2005a,b; Bogoevska *et al.*, 2006, 2007). In all cases, binding of DC-SIGN to its neutrophil counter-receptor was dependent on Le<sup>x</sup> epitopes that were expressed on these proteins. What effect DC-SIGN binding has on the neutrophil response is currently not well understood, however, it is known that ICAM-3, Mac-1 and CEACAM1 are involved in the regulation of neutrophil apoptosis (Yan *et al.*, 2004; Singer *et al.*, 2005; Kessel *et al.*, 2006). Therefore, DC-SIGN-dependent DC-neutrophil interactions may affect neutrophil survival (Ludwig *et al.*, 2006). This hypothesis is supported by the finding that the interaction of DCs with neutrophils prevents the downregulation of several markers, i.e. CD13, CD15, CD16 and Mac-1, on neutrophils (Megiovanni *et al.*, 2006). Downregulation of these markers is associated with increased neutrophil apoptosis (Dransfield and Rossi, 2004). Besides modulating neutrophil responses, DC-SIGN-neutrophil interactions also influence the DCs themselves. This

was demonstrated by the observation that neutrophil-induced DC maturation provokes a strong T-helper-1 cell (Th1-) polarized DC response (van Gisbergen *et al.*, 2005a). Collectively, these findings illustrate the importance of DC-SIGN in DC–neutrophil interactions. Furthermore, they show that these interactions influence both the neutrophil and the DC.

#### 4.4. DC-SIGN functions as an endocytic antigen receptor

The C-type lectins are well known for their capability to recognize antigens and mediate their uptake by antigen presenting cells. Besides the macrophage MR (Apostolopoulos and McKenzie, 2001), also DEC-205 (Mahnke *et al.*, 2000), BDCA-2 (Dzionic *et al.*, 2001), and DC-SIGN (Engering *et al.*, 2002a; Schjetne *et al.*, 2002) have been shown to perform such a function. By using anti-DC-SIGN monoclonal antibodies, it was demonstrated that DC-SIGN quickly internalizes upon antigen binding and subsequently traffics to late endosomal or lysosomal compartments where the complexes are degraded, loaded onto MHC complexes and presented to T-cells (Engering *et al.*, 2002a). In addition, DC-SIGN has been shown to mediate the recognition and uptake of various pathogens including human immunodeficiency virus (HIV)-1 and *Leishmania* amastigotes (Kwon *et al.*, 2002; Colmenares *et al.*, 2002). Notably, DC-SIGN endocytosis is dependent upon the dileucine motif in its cytoplasmic tail (Engering *et al.*, 2002a) (see Figure 34.1A). Whereas wild-type DC-SIGN was efficiently taken up, a mutated form, in which the leucines were replaced by alanines, endocytosed much less efficiently (Engering *et al.*, 2002a). In addition, DC-SIGN harbours a tri-acidic cluster: EEE (in one-letter amino-acid code) (see Figure 34.1A). For DEC-205, it has been shown that a comparable tri-acidic motif (EDE instead of EEE) mediates targeting to late endosomal compartments

(Mahnke *et al.*, 2000). Thus, in conclusion, DC-SIGN not only functions as an adhesion receptor but also mediates the uptake and presentation of pathogen-derived antigens by antigen presenting cells. While these functions suggest a primary role in host defence mechanisms, an increasing amount of evidence suggests that DC-SIGN–pathogen interactions may also be part of pathogenic strategies to increase the efficacy of infection and/or escape immune surveillance by modulating host immune responses.

## 5. PATHOGENS TARGET DC-SIGN TO SUBVERT HOST IMMUNE RESPONSES

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As discussed above, DC-SIGN functions as an antigen receptor that mediates the recognition and uptake of a wide variety of pathogens (see Table 34.1). The first pathogen that was shown to interact with DC-SIGN is HIV-1 (Geijtenbeek *et al.*, 2000c). It was demonstrated that binding of HIV-1 is mediated by the interaction between DC-SIGN and the HIV-1 glycoprotein gp120. Furthermore, it was shown that HIV-1 binding by DCs facilitates infection of HIV-1 permissive cells *in trans* (Geijtenbeek *et al.*, 2000c). Later on, several other viral pathogens were also shown to interact with DC-SIGN, demonstrating its function as a broad viral receptor. The interaction of DC-SIGN with viral pathogens and its role in viral infectivity has been the subject of many studies. Several high-quality reviews on DC-SIGN–virus interactions have recently appeared, including ones discussing the interaction of DC-SIGN with HIV-1 (Lekkerkerker *et al.*, 2006; Wu and KewalRamani, 2006), hepatitis C virus (Cocquerel *et al.*, 2006), Ebola virus (Baribaud *et al.*, 2002) and severe acute respiratory syndrome (SARS) corona virus (Chen and Subbarao, 2007). For this reason, DC-SIGN–virus interactions will not be discussed in further detail. Rather we will focus on the interaction

of DC-SIGN with another important class of pathogens, i.e. bacterial pathogens. In the next section, we will first discuss the ability of mycobacterial species to target DC-SIGN and thereby subvert DC functioning. After this, we continue with another pathogen, i.e. *Helicobacter pylori*, and see how this bacterium specifically modulates the Th1/Th2 balance through phase-variable interactions between its lipopolysaccharide (LPS) and DC-SIGN.

### 5.1. Mycobacteria target DC-SIGN to subvert DC functioning

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, is a major cause of death worldwide. The bacterium is transmitted through aerosols spread by people suffering from active clinical disease. After inhalation, *M. tuberculosis* infects alveolar macrophages in which it is able to persist for extensive periods of time. Normally, the infected macrophages are contained within so-called granulomas, however, in a substantial number of cases ( $\approx 10\%$ ), the bacterium is released from its containment and causes active disease (Russell, 2007). To date, the only licensed TB vaccine is the so-called Bacille Calmette-Guérin (BCG) vaccine. Although effective against disseminated TB in children, it does not protect well against pulmonary TB later in life. Moreover, the prevalence of multiple- and extensive drug-resistant *M. tuberculosis* strains increases each year.

The hallmark of mycobacterial disease is the bacterium's ability to persist in host tissues for many years. This feature, which is also referred to as latency, is dependent on at least two different processes. The first one is the ability of the bacterium to inhibit phago-lysosome fusion. Normally, ingested bacteria are contained within phagosomes which later on fuse with lysosomes leading to bacterial destruction and subsequent presentation on MHC complexes. However, pathogenic mycobacteria interfere with this process

by actively blocking phago-lysosome fusion thereby creating a unique niche in which they are able to survive for many years (Rohde *et al.*, 2007). For the past decades, the central dogma has been that pathogenic mycobacteria exclusively reside within this phagosomal compartment until they are released from the cell and cause active disease. However, recently, this view was challenged by the observation that *M. tuberculosis*-containing phagosomes rapidly fused with lysosomes in both monocyte-derived DCs and macrophages (van der Wel *et al.*, 2007). Yet, at day 2 post infection, *M. tuberculosis* was able to escape from the phago-lysosomes into the cytosol where they were able to replicate (van der Wel *et al.*, 2007). This same phenomenon was observed for *Mycobacterium leprae* but not for the vaccine strain *Mycobacterium bovis* BCG or for heat-killed mycobacteria, suggesting that the process is specific for pathogenic mycobacteria. Although these findings contradict earlier observations and await independent confirmation, they may have important implications for the current view on host-mycobacterial interactions.

A second feature that supports the ability of pathogenic mycobacteria to cause chronic infections is their capacity to suppress host immune responses. To this end, DCs form an interesting target due to their central role in the induction of adaptive immunity. Although early data already indicated that mycobacteria influence DC functioning, the underlying mechanisms remained poorly understood. Major progress into this field was made when it was found that the interaction between *M. tuberculosis* and DCs is almost exclusively dependent on the binding to DC-SIGN (Geijtenbeek *et al.*, 2003; Tailleux *et al.*, 2003) (see also Chapter 9). Although DCs express a wide variety of C-type lectins, such as the MR and dectin-1, only antibodies directed against DC-SIGN could block the *Mycobacterium*-DC interaction (Tailleux *et al.*, 2003). Comparable results were obtained with the *M. bovis* BCG vaccine strain (Tailleux *et al.*, 2003; Geijtenbeek and van Kooyk, 2003).

In an effort to identify the ligand responsible for binding to DC-SIGN, it was found that binding to *M. tuberculosis* could almost completely be inhibited by pre-incubating DC-SIGN with Man-capped lipoarabinomannan (ManLAM), a major glycolipid of the *M. tuberculosis* cell wall, suggesting that this component forms an important ligand for DC-SIGN (Tailleux *et al.*, 2003). Moreover, it was shown that the interaction of ManLAM with DC-SIGN was dependent on the terminal Man caps as AraLAM, an uncapped form of ManLAM, did not bind to DC-SIGN (Geijtenbeek *et al.*, 2003). Later on, these findings were confirmed by the demonstration that DC-SIGN specifically interacts with neo-glycoconjugates that resemble the Man-cap of ManLAM (Koppel *et al.*, 2004). Furthermore, it was shown that a reduction of the number of mannosyl residues in the cap-derived neo-glycoconjugates leads to a decreased affinity for DC-SIGN (Koppel *et al.*, 2004). Interestingly, ManLAM is exclusively found in pathogenic, slow-growing mycobacteria, whereas AraLAM is only found in avirulent, fast-growing species, suggesting that ManLAM-DC-SIGN interactions may be important for mycobacterial pathogenesis. These data, together with the finding that also PI-LAM, a phosphoinositide-capped form of LAM, poorly inhibits DC-SIGN-*M. tuberculosis* interactions, led to the hypothesis that DC-SIGN can discriminate between mycobacterial species through the recognition of the Man caps on LAM (Maeda *et al.*, 2003). However, later on, this view was found to be too simplistic as it was demonstrated that not all mycobacterial species that contain ManLAM are bound by DC-SIGN (Pitarque *et al.*, 2005). This was not due to intrinsic differences between the different ManLAMs, as it was shown that the purified ManLAMs, also the ones from the species that did not bind DC-SIGN, could all efficiently block DC-SIGN-*M. tuberculosis* interactions (Pitarque *et al.*, 2005). Furthermore, experiments addressing the cell-surface exposure of ManLAM showed that between species only minor differences

exist (Pitarque *et al.*, 2005). These data strongly suggested that ManLAM was not the only DC-SIGN ligand present on mycobacteria and further investigations led to the discovery of at least four additional ligands: lipomannan, Man-capped arabinomannan and two mannosylated glycoproteins (19 and 45 kDa antigens) (Pitarque *et al.*, 2005). In a later study, also phosphatidylinositol-mannosides were shown to bind to DC-SIGN (Torrelles *et al.*, 2006).

The strongest evidence that the presence of Man caps on LAM is not essential for the binding of mycobacteria to DC-SIGN was provided in a study using *Mycobacterium marinum* and *M. bovis* BCG strains devoid of Man caps on LAM (Appelmelk *et al.*, 2008). It was demonstrated that the mutant strains bound as efficiently to DC-SIGN as the wild-type strains. Furthermore, the interaction could still be blocked with mannan. In addition, the mutants did not show any significant differences in *in vivo* survival and induced similar cytokine profiles (Appelmelk *et al.*, 2008). The only difference observed was that the phagocytosis of the capless *M. marinum* strain by macrophages was slightly reduced and that the mutant induced somewhat more phagosome-lysosome fusion, which is consistent with the earlier finding that ManLAM inhibits this process (Fratti *et al.*, 2003; Hmama *et al.*, 2004). Overall, this study demonstrated that the Man caps of LAM do not dominate the *Mycobacterium*-host interaction. This finding was unexpected since ManLAM was not only shown to bind to DC-SIGN but was also known to modulate DC cytokine secretion by suppressing the production of IL-12 and/or upregulating the production of IL-10, thereby inhibiting Th1-type immune responses (Nigou *et al.*, 2001; Geijtenbeek *et al.*, 2003). Interestingly, Geijtenbeek *et al.* (2003) observed that ManLAM, but not AraLAM, induces IL-10 secretion in LPS-primed DCs. By using monoclonal antibodies, they demonstrated that the phenomenon was dependent on DC-SIGN, suggesting that DC-SIGN ligation can modulate TLR responses (Geijtenbeek *et al.*, 2003).

Recently, the molecular signalling pathway underlying this modulatory activity was identified (Gringhuis *et al.*, 2007). It was demonstrated that pathogens target DC-SIGN to activate the serine/threonine kinase Raf-1, which subsequently leads to acetylation of the nuclear factor-kappaB (NF- $\kappa$ B) subunit p65, but only after NF- $\kappa$ B has been activated via TLRs. Acetylation of p65 both prolonged and increased *IL-10* transcription to enhance the DCs anti-inflammatory cytokine responses (Gringhuis *et al.*, 2007). These results demonstrate that the role of DC-SIGN in host-pathogen interactions can be interpreted in two ways. On the one hand, DC-SIGN forms an important pathogen receptor that mediates the uptake and destruction of a large panel of pathogenic microorganisms. Yet, on the other hand, pathogens can also exploit DC-SIGN to modulate DC responses and thereby subvert host immunity. The potential importance of DC-SIGN ligation during host infection is illustrated by the observation that microorganisms, such as mycobacteria, may express a large number of DC-SIGN ligands. Furthermore, pathogens may actively induce the expression of DC-SIGN. Interestingly, in the case of *M. tuberculosis*, it has been shown that in patients with TB, up to 70% of alveolar macrophages express DC-SIGN. By contrast, the lectin was hardly detected in alveolar macrophages from healthy individuals or in patients with unrelated lung pathologies (Tailleux *et al.*, 2005). Moreover, promoter polymorphisms that influence DC-SIGN expression have been associated with an altered susceptibility to mycobacterial infections (Barreiro *et al.*, 2006; Vannberg *et al.*, 2008).

## 5.2. *H. pylori* modulates Th1/Th2 polarization through interactions with DC-SIGN

*H. pylori* is a common human pathogen that has persistently colonized about 50% of the total human population. Colonization typically

occurs early in life and may persist during the host's entire lifetime. In most cases, the infection is asymptomatic, however, in about 10% of the cases, the infection leads to disease, which can range from relatively mild gastritis and peptic ulcers to life-threatening diseases such as gastric cancer (Ernst and Gold, 2000; Makola *et al.*, 2007). *H. pylori* expresses a wide variety of virulence factors including LPS. In general, LPS is an amphiphilic molecule that is located in the outer membrane of Gram-negative bacteria. It consists of three distinct domains: lipid A, a core oligosaccharide and an O-antigen (Raetz and Whitfield, 2002) (see Chapters 3 and 4). Although LPSs are considered to have a strong immunostimulatory activity which is primarily dependent on the interaction between lipid A and TLR-4 (Palsson-McDermott and O'Neill, 2004), *H. pylori* lipid A is unusual in that it contains a variety of modifications that abrogate the interaction with TLR-4, thereby lowering the endotoxic activity of the LPS (Moran, 2007). The *H. pylori* core oligosaccharide consists of an inner core of a 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) moiety substituted with a linear heptose tetrasaccharide and an outer core encompassing Gal and glucose residues (Aspinall *et al.*, 1996; Aspinall and Monteiro, 1996). The O-antigen is linked to the heptose core and is composed of a poly-(*N*-acetyl- $\beta$ -lactosamine) chain decorated at some positions with L-Fuc residues to produce internal Le<sup>x</sup> determinants with terminal Le<sup>x</sup> or Le<sup>y</sup> moieties (Aspinall *et al.*, 1996; Aspinall and Monteiro, 1996). Furthermore, some strains may express Le<sup>a</sup>, Le<sup>b</sup>, Le<sup>c</sup> and sialyl-Le<sup>x</sup> units, as well as H-1 and blood groups A and B antigens, giving rise to a large variation in LPS-core composition (Moran, 2008). Importantly, Le<sup>x</sup> and Le<sup>y</sup> expression is a common property of *H. pylori* strains and it is found in 80–90% of all cases. Yet, expression of Le epitopes can vary within a single strain as a consequence of the phase variable expression of the responsible fucosyltransferases, i.e. FutA, FutB and FutC (Wang

*et al.*, 2000; Bergman *et al.*, 2006; Sanabria-Valentin *et al.*, 2007). The frequency of “on-off” switching of Le antigen expression has been reported to be between 0.2 and 0.5% (Appelmelk *et al.*, 1998). Biologically, Le epitope expression is thought to mediate the evasion of host immune responses and influence bacterial colonization and adhesion (Moran, 2008). Furthermore, Le antigen expression has been shown to mediate the binding of *H. pylori* to DC-SIGN (Bergman *et al.*, 2004). Whereas strains harbouring Le<sup>x</sup> and Le<sup>y</sup> epitopes strongly bound to DC-SIGN, strains devoid of these structures did not (Bergman *et al.*, 2004). Interestingly, it was demonstrated that the Le-dependent interaction with DC-SIGN inhibited Th1 responses by inducing the production of IL-10 (Bergman *et al.*, 2004). Thus, since Le expression is phase variable, a typical *H. pylori* population will contain a mixture of DC-SIGN-binding and non-binding bacteria. Bacteria unable to bind DC-SIGN will primarily induce a Th1-type response. Yet, this Th1 polarization is counterbalanced by bacteria that target DC-SIGN, thereby leading to a mixed Th1/Th2 response. It is hypothesized that the induction of a mixed response promotes the establishment of chronic infections. This is supported by the observation that people suffering from chronic gastritis display a combined secretion of both Th1 and Th2 cytokines (D’Elios *et al.*, 2005). Furthermore, it is known that Th1 polarization of *H. pylori*-specific T-cell responses is associated with more severe disease (D’Elios *et al.*, 2005).

## 6. CONCLUSIONS

Ever since its discovery, DC-SIGN has received major scientific attention. It is now clear that the biological role of DC-SIGN is two-fold. On the one hand, DC-SIGN fulfils important functions necessary for the induction of successful immune responses that are essential for the clearance of microbial infections. Yet,

on the other hand, pathogens may also exploit DC-SIGN to modulate DC functioning thereby skewing the immune response and promoting their own survival. Currently, a lot is known about the structure and carbohydrate specificity of DC-SIGN and crystallographic studies have provided us with detailed insights into the molecular mechanisms underlying DC-SIGN-carbohydrate interactions. However, much less is known about the role of DC-SIGN during *in vivo* infection. Obviously, there are good indications that suggest it plays an important role, however, these are mostly based upon *in vitro* experiments. Hence, one important goal is to obtain insight into the role of DC-SIGN during infection and see how it functions within the complexity of the immune system.

To enlighten this issue, the use of appropriate animal models seems essential. Nevertheless, one major problem is that, although DC-SIGN homologues can be found in many organisms, their carbohydrate specificity, expression pattern and immunological function may vary significantly. One example is the murine model. Like humans, mice express a set of DC-SIGN-like molecules, i.e. mDC-SIGN and mSIGNR1-4, of which some (mSIGNR1 and mSIGNR3) share a carbohydrate specificity similar to human DC-SIGN (Galustian *et al.*, 2004). Yet, unlike human DC-SIGN, mSIGNR1 is not expressed by DCs and mSIGNR3 only at low levels (Koppel *et al.*, 2005), suggesting that these molecules exhibit distinct functions and, thus, that the murine model is unsuitable for studying the role of human DC-SIGN. One possibility is to use primates as these animals possess DC-SIGN homologues that resemble both the specificity and expression pattern of human DC-SIGN. However, due to both ethical and technical difficulties their use may not be feasible. A second option is to make use of humanized model systems. By reconstituting mice with human immune cells or by heterologously expressing human DC-SIGN in murine strains, it may be possible to study DC-SIGN in *in vivo* relevant

situations. Both these approaches have recently been applied to study the function of DC-SIGN (Kretz-Rommel *et al.*, 2007; Schaefer *et al.*, 2008).

Another important goal is to dissect further the molecular signalling cascades downstream of DC-SIGN. It is clear that DC-SIGN can interfere with TLR signalling. It has been shown that DC-SIGN directly influences the acetylation of NF- $\kappa$ B p65 via Raf-1. Nonetheless, it remains unclear whether this route is the only way by which DC-SIGN influences TLR-signalling or whether alternative regulatory mechanisms may exist. Furthermore, it is unclear whether DC-SIGN can also influence unrelated signalling pathways. Moreover, it will be interesting to see whether distinct DC-SIGN ligands all function in a similar way.

The increasing knowledge on DC-SIGN has also led to some, potentially, interesting applications. One idea is that DC-SIGN can be used to target antigens specifically to DCs, thereby generating a more efficient immune response. The feasibility of such an approach was recently demonstrated when it was shown that glycan modification of the tumour antigen gp100 targets it to DC-SIGN and enhances DC-induced antigen presentation to T-cells (Aarnoudse *et al.*, 2008). Furthermore, it has been shown that administration of anti-DC-SIGN antibodies fused with either tetanus toxoid peptides or keyhole limpet haemocyanin raised efficient T-cell responses without additional adjuvant requirements (Kretz-Rommel *et al.*, 2007). Therefore, targeting of antigens

to DC-SIGN may be a promising strategy to induce enhanced immune responses against both cancer and microbial antigens. Currently, the picture is emerging that pathogens target DC-SIGN specifically to suppress Th1 immunity, as exemplified by the observations that both mycobacterial ManLAM and *H. pylori* LPS suppress IL-12 secretion and/or induce IL-10 production by immune cells. In principle, this knowledge can be used to counteract the bacteria as the removal of DC-SIGN ligands may thus lead to improved Th1 responses and, thereby, enhance the efficacy of certain vaccines. One important vaccine to which this approach may apply is the BCG vaccine which is currently used to prevent *M. tuberculosis* infection. This vaccine is effective in preventing disseminated TB in children, but is poorly able to prevent pulmonary TB later in life. The general idea is that the BCG vaccine induces a sub-optimal immune response thereby abrogating protective immunity. As DC-SIGN seems to be involved in mycobacterial immunosuppression, it would be interesting to see whether the removal of mycobacterial DC-SIGN ligands can increase the potency of BCG vaccines.

Overall, the discovery of DC-SIGN has led to important insights into the interactions between pathogens and their hosts. Yet, important questions remain to be resolved (see Research Focus Box). Nevertheless, DC-SIGN forms an interesting target that may pave the way for the design of new therapeutic approaches against both microbial infections and cancer.

#### RESEARCH FOCUS BOX

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- Why does DC-SIGN bind to Le<sup>a</sup> and Le<sup>y</sup> but not to the closely related Le<sup>x</sup> structure?
- How important is ICAM-3 in DC-SIGN-dependent DC and T-cell interactions?
- What is the functional role of DC-SIGN splice variants?
- How important is DC-SIGN ligation during *in vivo* infection?

## RESEARCH FOCUS

## BOX (cont'd)

- Do all DC-SIGN ligands behave similarly in terms of induced downstream signalling cascades?
- Does DC-SIGN ligation modulate other host cell responses than IL-10/IL-12 cytokine production?
- How important are DC-SIGN-related polymorphisms for susceptibility to and outcome of microbial infections?
- How feasible is the approach to target DC-SIGN for the development of novel therapeutics against microbial infections, cancer and, maybe, autoimmune diseases?

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