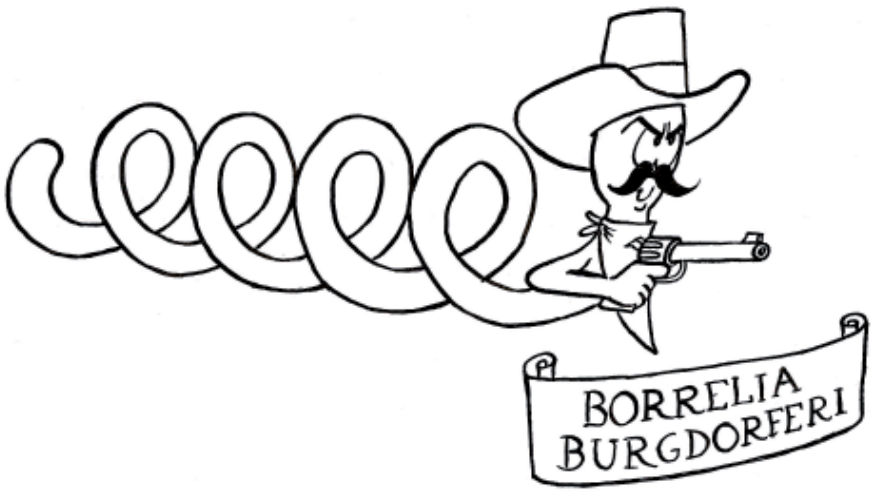
Chapter 1



General Introduction

Dendritic cells and immunity against pathogens

Evolution has equipped humans with a highly effective immune system against pathogens, which uses the two-armed system of innate and adaptive immunity to eliminate infectious microorganisms. The innate immune system provides a first line of defense, with macrophages and neutrophils that phagocytose and kill pathogens through the production of antimicrobial agents. On the other hand, T and B cells comprise the adaptive immune system, which recognizes pathogens through antigen-specific interactions and generates immunological memory to enable a quick response upon a secondary encounter with a pathogen. At the bridge linking these two arms of the immune system stand the antigen presenting cells (APCs), which recognize pathogens and subsequently efficiently stimulate naïve T cells, thereby coupling initial pathogen recognition to adaptive immunity. Although macrophages and B cells can act as APCs, the most important APCs are the highly specialized dendritic cells (DCs).

DCs are a heterogeneous population of cells that are localized underneath epithelia throughout the body, thereby ideally positioned to intercept invading pathogens. DCs reside in peripheral tissues in an 'immature' state. Sensing and capturing a pathogen induces DC maturation, which mediates several changes in DC behavior that are vital for the generation of effective T cell responses. Due to a switch in the chemokine receptor profile, mature DCs migrate from peripheral tissues to draining lymph nodes for the interaction with recirculating T cells [1]. DCs efficiently load peptides from internalized pathogenic antigens on MHC molecules for presentation to T cells [2, 3]. In addition, mature DCs upregulate co-stimulatory molecules such as CD80 and CD86 to provide additional signals required for efficient induction of effector T cells [4, 5].

Moreover, DCs tailor adaptive immune responses to the type of pathogen involved for rapid and efficient clearance of infection. Effective immunity against intracellular or extracellular pathogens requires different types of immune responses. In order to adequately cope with these different pathogens, DCs mediate the differentiation of naïve T helper cells along distinct pathways. To date, four distinct T helper cell subsets have been identified, which include T helper (T_H)1, T_H2, T_H17 and regulatory T helper (T_{reg}) cells. T_H1 cells produce interferon (IFN)-γ and their primary role is to protect against intracellular microbes via cell-mediated immunity and the production of opsonizing antibody classes [6]. T_H2 cells produce interleukin (IL)-4, IL-5, IL-9 and IL-13 and are involved in protection against extracellular pathogens such as gastrointestinal nematodes through the induction of humoral immunity [6]. Recently, a novel class of CD4+ T helper cells was identified, which is characterized

by IL-17 production and was therefore named T_H17 [7, 8]. Although its role in immunity against pathogens has not been completely unraveled, there is increasing evidence that T_H17 cells play a role in protection against bacterial and fungal pathogens by promoting pro-inflammatory cytokines and chemokines resulting in recruitment and activation of neutrophils and macrophages [9]. T_{reg} produce IL-10, transforming growth factor (TGF)β and IL-35 and are essential for limiting excessive immune activation by mediating immune suppression [10].

The induction of specific classes of T helper cells is regulated by the production of specific cytokines by DCs [9, 11, 12] (Figure 1): IL-12 promotes the development of T_H1, while IL-4 promotes T_H2 development [11]; a combination of several cytokines including TGFβ, IL-1β, IL-6, IL-21 and IL-23 are involved in the development of T_H17 [9]; and TGFβ and IL-10 induce T_{reg} development [13]. For the induction of these pathogen-specific cytokine profiles, DCs are equipped with several pathogen-sensing receptors [14]. These receptors recognize the specific type of pathogen through signature structures, resulting in the induction of signaling pathways that generate a pathogen-specific cytokine profile and a consequential T helper cell response. Therefore, pathogen recognition through these receptors is crucial for effective adaptive immunity.

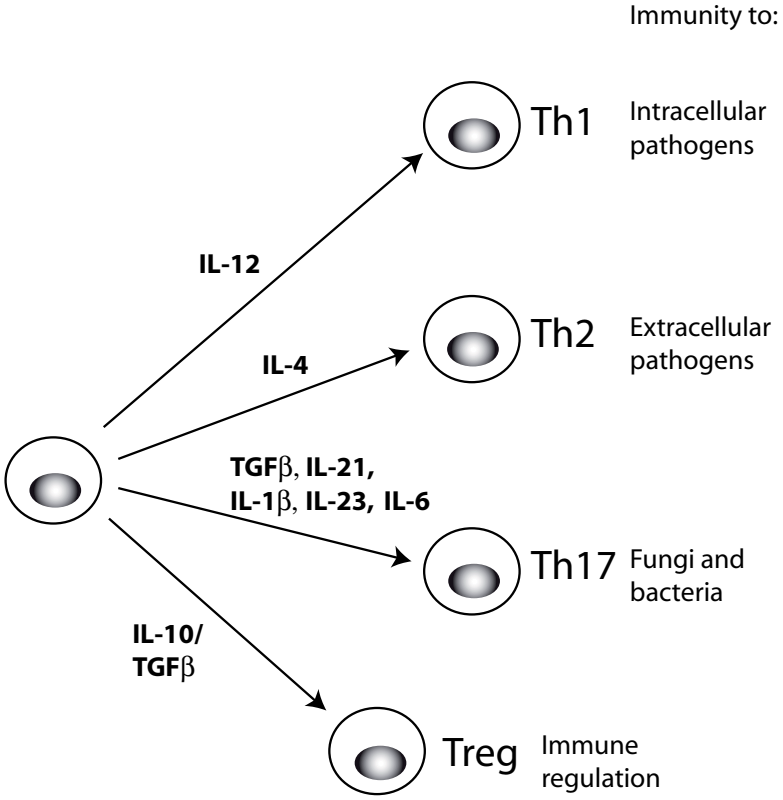


Figure 1 Induction of different subtypes of T helper cells. Naïve T helper cells differentiate into T_H1, T_H2, T_H17 or T_{reg} under the influence of specific cytokines produced by DCs. IL-12 induces the development of T_H1 cells, which mediate immunity to intracellular pathogens. IL-4 induces T_H2 development, which mediates immunity to extracellular pathogens. T_H17 development is promoted by combinations of various cytokines including TGFβ, IL-21, IL-1β, IL-6 and IL-23, which mediates immunity against fungal and bacterial pathogens. IL-10 and TGFβ promote the induction of inducible T_{reg} cells, which regulate immune responses by limiting collateral damage during immune responses to pathogens.

Pattern Recognition Receptors

Immature DCs sense pathogens via various surface, endosomal and cytoplasmic proteins collectively referred to as pattern-recognition receptors (PRRs). These PRRs recognize so-called pattern associated molecular patterns (PAMPs), which are conserved groups of molecules from pathogens that are essential for microbial survival, such as bacterial or fungal cell wall components and viral or bacterial nucleic acids [15]. In the broadest sense, PRRs comprise any PAMP receptor that is capable of inducing antimicrobial effects in leukocytes, including processes such as phagocytosis or the release of cytotoxic granules [16]. As a more restricted definition, PRRs are PAMP-receptors that transmit intracellular signals that regulate the expression of response genes, such as those encoding co-stimulatory molecules and cytokines and chemokines. Induction of those genes is essential not only for early protection against pathogen intrusion, but also for coupling innate with adaptive immunity [17].

The repertoire of PRRs that can regulate gene expression on DCs is restricted to just a few classes (Figure 2). The best-studied PRRs are the family of Toll-like receptors (TLRs), which in human comprises ten different types that are expressed on the cell surface (TLR 1/2/4/5/6) or in endosomal compartments (TLR 3/7/8/9) [18]. TLRs detect key components of many bacteria and viruses including CpG DNA, RNA as well as lipopolysaccharide (LPS). TLRs initiate signal transduction pathways via adaptor molecules such as myeloid differentiation response gene 88 (MyD88) and/or TIR-containing adaptor-inducing IFN-β (TRIF), resulting in activation of several pathways including nuclear factor-κB (NF-κB) and mitogen activated protein (MAP) kinases [19].

NOD-like receptors (NLRs) represent a family of cytosolic PRRs that are important in the recognition of intracellular bacteria [20, 21]. NLRs consist of more than 20 structural related molecules that recognize bacterial products such as peptidoglycan. NLRs can be grouped into molecules that contain either a caspase-recruitment domain (Card) or a Pyrin motif [22]. NOD proteins mediate the activation of NF-κB. In addition, Pyrin expressing proteins regulate the activation of inflammatory caspases within a multiprotein complex known as the 'inflammasome', which leads to the cleavage and activation of pro-inflammatory cytokines such IL-1β and IL-18.

A third class of PRRs are the retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), which are essential for viral recognition in the cytoplasm and are comprised of RIG-I, melanoma differentiation-associated gene-5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) [23]. Recognition of viral RNA by RIG-I and MDA5 induces conformational changes that allow the interaction with adaptor protein IFN promoter stimulator-1

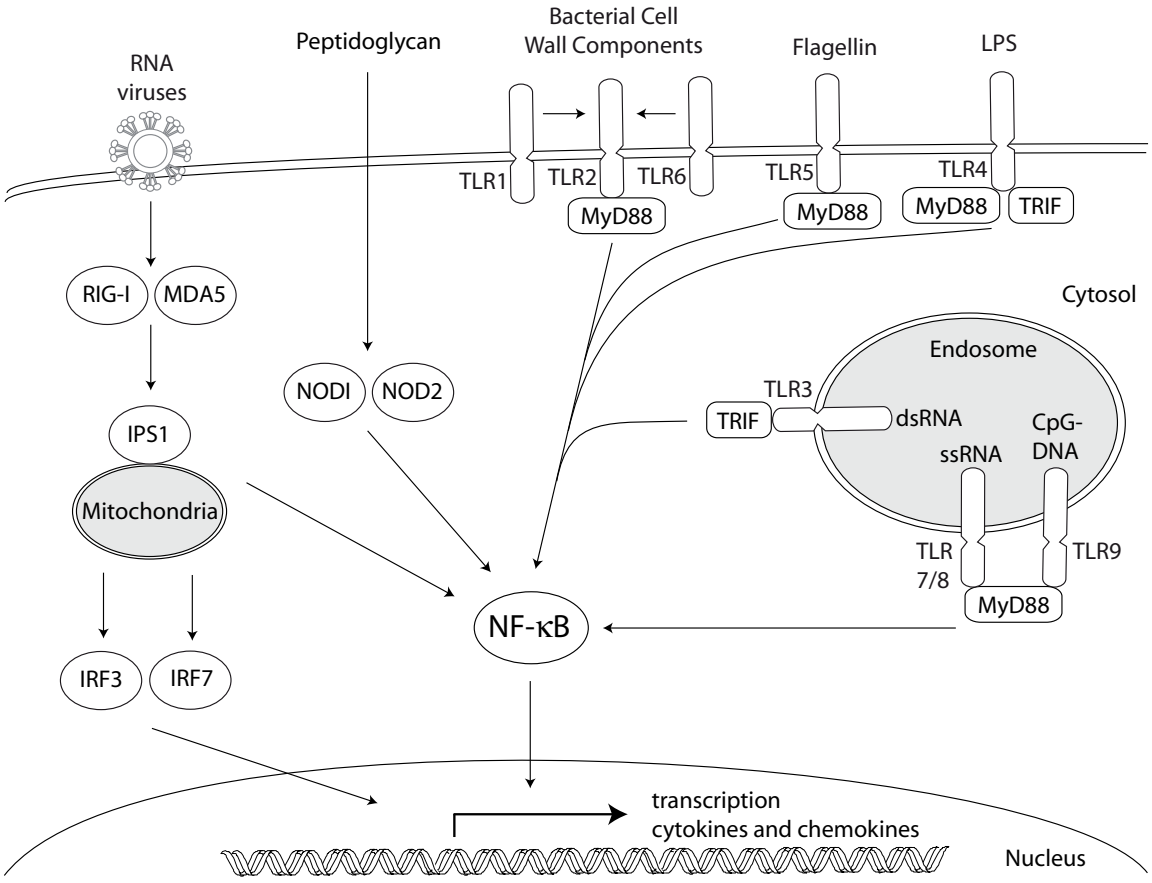


Figure 2 PRRs that induce the transcription of innate response genes. TLRs are expressed on the cell-surface and in endosomal compartments and detect components of various classes of pathogens including viruses, bacteria and fungi. NLRs are cytosolic PRRs that are important for detecting intracellular bacteria. RLRs are essential for viral recognition in the cytoplasm. Signaling via these receptors leads to the induction cytokines and chemokines via activation of transcription factors such as NF-κB.

(IPS-1) on mitochondria. Subsequent downstream signaling leads to activation of interferon regulatory factor 3 and 7 (IRF-3 and -7) and NF-κB, which are critical for antiviral immunity by inducing the production of type I interferons and several other pro-inflammatory cytokines.

Notably, most pathogens express different PAMPs and therefore interact with DCs through several classes of PRRs simultaneously. As a result, the ultimate expression of response genes, and consequently the polarization of immune responses against a given pathogen, depends on the cross-talk and integration of the different signaling pathways of the PRRs.

C-type lectins

In addition to the previously described receptors, C-type lectins have in recent years emerged as PRRs that play an essential role in induction of immune responses against numerous pathogens (for an overview of C-type lectins that interact with pathogens see Figure 3). The C-type lectin family encompasses a large family of proteins that besides DCs are also expressed by other cell types such as macrophages, NK cells and endothelial cells [24]. DCs express several C-type lectins including dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN), dendritic cell-associated C-type lectin-1 and 2 (dectin-1 and 2), mannose receptor (MR), Langerin, dendritic cell receptor 205 (DEC-205) and macrophage galactose-type lectin (MGL). However, expression of C-type lectins is in some cases confined to specific subsets of DCs, such as Langerin on human epidermal Langerhans cells (LCs) [25] and blood dendritic cell antigen-2 (BDCA-2) on plasmacytoid DCs (pDCs) in blood [26]. C-type lectins are classified based on the expression of a structural motif in their carbohydrate recognition domain (CRD) [27]. Although originally its name denoted calcium-dependent carbohydrate recognition, the C-type lectin family now also includes several proteins without obvious calcium binding or carbohydrate specificity. Most C-type lectins expressed by DCs are type II transmembrane proteins (external C-terminus and internal N-terminus) that express a single CRD. In contrast, the MR and DEC205 are type I transmembrane proteins (external N-terminus and internal C-terminus) that express eight and ten CRDs respectively.

Different C-type lectins recognize distinct carbohydrate structures, which is related to the amino acid sequences in their CRD. The presence of a Glu-Pro-Asn (EPN) site in the CRD predicts specificity for mannose and/or fucose terminated glycans, while a Gln-Pro-Asp (QPD) site determines specificity for galactose-containing carbohydrates [28-30]. A secondary binding site, which is located adjacent to the primary binding site of the CRD and interacts with neighbouring residues in the interacting carbohydrate structure, fine-tunes the carbohydrate-specificity so that each C-type lectin has its own unique glycan specificity [31]. In addition, C-type lectins can form oligomers, which not only enhances the affinity, but also favors the binding of carbohydrates with a specific density and spacing [32]. As a result, C-type lectins that have similar basic specificities for mannose can still interact with a very diverse set of ligands.

C-type lectins on DCs mediate a diverse range of functions. Besides the recognition of pathogens, C-type lectins also mediate processes via recognition of glycosylated self-antigens. For example, DC-SIGN recognizes self-glycoproteins intercellular adhesion molecule 2 (ICAM-2) and ICAM-3 that regulate DC migration [33] and DC-T cell interaction [34]

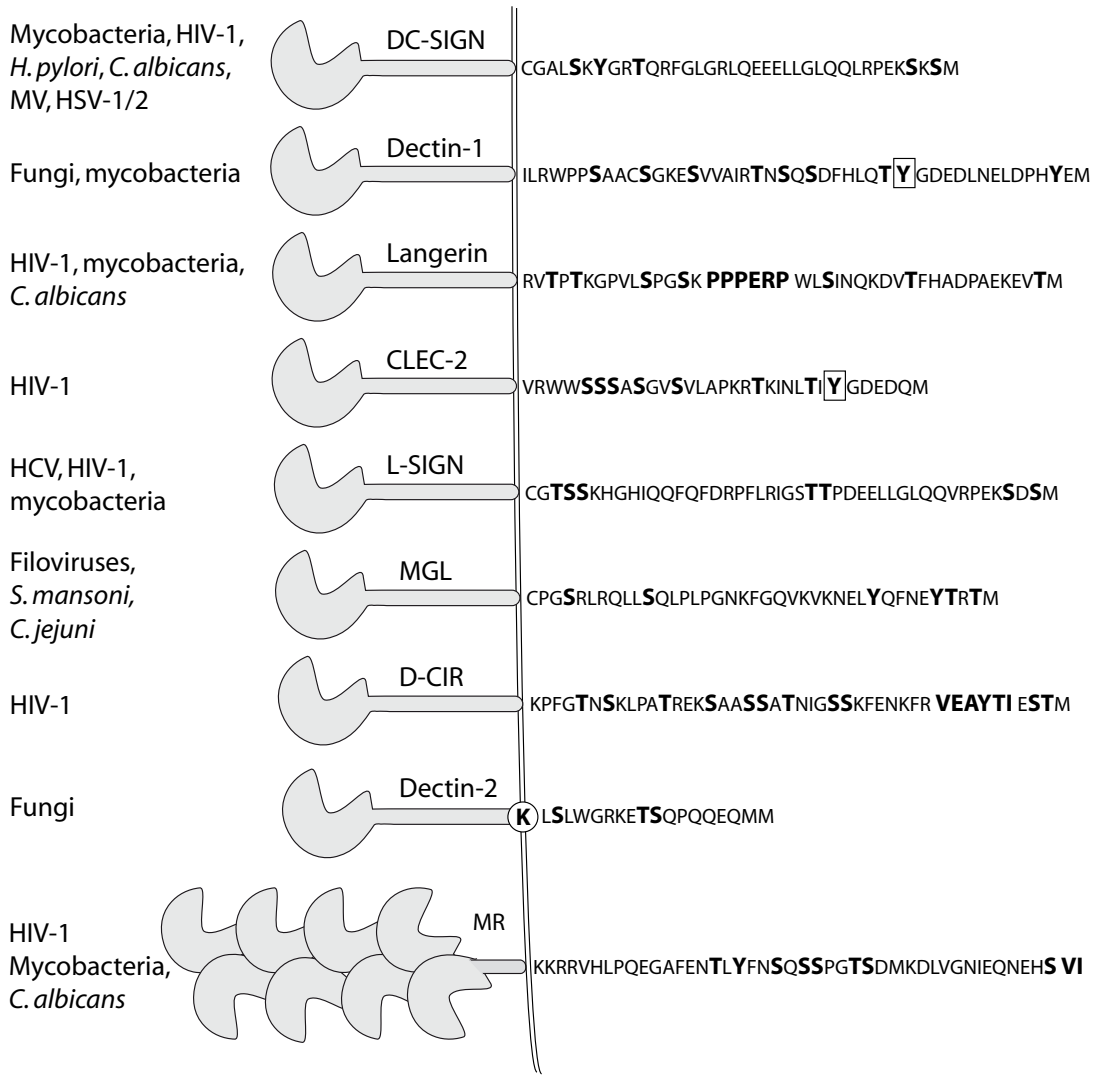


Figure 3 Overview of C-type lectins that interact with pathogens. Based on their carbohydrate recognition profile, C-type lectins recognize distinct pathogens. C-type lectins display several residues in their cytoplasmic tails that might be responsible for coupling of pathogen recognition to intracellular signaling: D-CIR contains an ITIM motif; Langerin contains a proline rich stretch that can be targeted by SH3 domain containing proteins; some receptors contain tyrosine (Y) residues and nearly all receptors contain serine (S) and threonine (T) residues, which upon phosphorylation become potential docking sites for signaling proteins. Dectin-2 contains a positively charged residue (K; lysine) within the transmembrane region, which might mediate the association with other proteins containing a negatively charged residue; the MR contains hydrophobic residues at its C-terminus, which could be bound by PDZ domain-containing proteins. The tyrosine residues of dectin-1 and CLEC-2 (boxed) have been identified to be crucial for signaling by these C-type lectins.

respectively. In addition, C-type lectins bind to and internalize pathogens for processing and antigen presentation, thereby initiating immune responses against these microorganisms. To internalize pathogens most C-type lectins express putative internalization motifs in their cytoplasmic domain such as a di-leucine motif (Leu-Leu) and a tri-acidic cluster (Glu-Glu-Glu) [35]. Some C-type lectins target internalized antigens to lysosomes and MHC class II-positive late endosomes [36-38], while other C-type lectins,

such as the MR, DEC-205 and ASGPR recycle back to the cell surface via early endosomes, thereby mediating large amounts of antigen uptake [39].

While C-type lectins have been known for years to be involved in intercellular interactions and uptake of self and non-self antigens, it has only recently been recognized that C-type lectins in response to pathogens can also induce gene transcription. As suggested by the several potential signaling motifs in their cytoplasmic domains (Figure 3), the interaction of C-type lectins with pathogens can directly lead to the induction of intracellular signaling cascades that direct immune responses. DC-SIGN and dectin-1 are two C-type lectins in particular that have been identified to couple pathogen recognition to induction and modulation of gene transcription. The intracellular signaling pathways induced by these C-type lectins modulate the responses of other PRRs such as TLRs, but also exert functions independent from other PRRs [40-43]. The role of DC-SIGN and dectin-1 as PRRs is amplified in the next two paragraphs.

DC-SIGN

DC-SIGN is a calcium-dependent C-type lectin expressed by DCs [34] and macrophage subpopulations [44] that mediates a wide range of immunological functions. DC-SIGN bears the EPN motif in its CRD, which determines its affinity for mannose- and fucose-containing carbohydrates [27]. DC-SIGN binds both complex mannose-containing glycoconjugates and fucose-containing carbohydrate structures such as Lewis blood-group antigens (Le^X, Le^Y, Le^A, Le^B) [45]. DC-SIGN contains seven complete and one incomplete tandem repeats within its neck-domain that may enable multimerization. Indeed, DC-SIGN forms tetramers, which may strengthen interactions by enabling high avidity binding [46, 47]. The carbohydrate recognition pattern is the basis of its specificity for a plethora of self and non-self antigens. As mentioned before, DC-SIGN acts as an adhesion receptor that interacts with self antigens ICAM-2 on endothelial cells to induce tethering and trans-endothelial migration of DCs [33] and mediates clustering of DCs with naive T cells through binding of ICAM-3 [34]. In addition, the carbohydrate specificity of DC-SIGN allows it to interact with a large array of pathogens [48]. DC-SIGN recognizes several classes of pathogens including viruses such as human immunodefiency virus 1 (HIV-1) [49] and measles virus (MV) [50], (myco)bacteria such as *Mycobacterium tuberculosis* [51, 52] and *Helicobacter pylori* [45, 53], and yeasts such as *Candida albicans* [54] and *Saccharomyces cerevisiae* [34].

Pathogen binding to DC-SIGN mediates diverse effects. Interaction

of DC-SIGN on DCs with HIV-1 has been shown to play an important role in promoting systemic dissemination of HIV in the host [49]. DCs located underneath the mucosal epithelium at sites of HIV infection can capture HIV-1 through high-affinity interaction of DC-SIGN with gp120, the HIV-1 envelope glycoprotein. After capture HIV-1 is internalized into non-lysosomal compartments, thereby allowing escape from degradation. Subsequently, DCs travel to the lymph node, where they transmit the virus to CD4+ T cells, the primary target cells of infection, through the formation of a so-called infectious synapse between HIV-1-infected DCs and CD4+ T cells [49, 55]. DC-SIGN also mediates this so called 'trans' infection for several other viruses including MV [50], HCMV [56], HCV [57], HSV-1 [58], Dengue [59] and Ebola virus [60], although some of these viruses also directly infect DCs through interactions with DC-SIGN [50, 56, 59-61].

Besides being a receptor that promotes viral transmission, DC-SIGN functions as a PRR that modulates immune responses against pathogens by directing the transcription of response genes. Remarkably, DC-SIGN does not induce gene transcription in its own, but instead DC-SIGN modulates cytokine and chemokine induction induced by other PRRs, such as TLRs. In addition, the way in which DC-SIGN modulates immune responses depends on the pathogen involved. Mycobacteria such as *M. tuberculosis* bind to DC-SIGN via ManLAM, which is abundantly expressed in mycobacterial cell walls, but is also secreted by *M. tuberculosis*-infected cells [62-64]. Binding of ManLAM to DC-SIGN alone does not induce cytokine production by DCs. However, binding of ManLAM to DC-SIGN does enhance the production of the immunosuppressive cytokine IL-10 induced by TLR activation [51]. Similarly, the human gastric pathogen *H. pylori* targets DC-SIGN to enhance IL-10 production. *H. pylori* spontaneously switches on and off Lewis antigens on LPS, a process known as phase-variable expression, which results in Lewis+ and Lewis- bacteria within a single strain [65]. Binding of Lewis+ antigens from *H. pylori* to DC-SIGN on DCs enhances IL-10 production and inhibits T_H1 polarization [53]. In contrast, binding of LPS from mutants of *Neisseria meningitidis* to DC-SIGN skews T cells towards a T_H1 response [66]. In addition, DC-SIGN binding by specific *Lactobacilli* species induces regulatory T cell differentiation [67]. As binding of DC-SIGN by different pathogens results in distinct immunological outcomes, these findings support an important role for DC-SIGN as an immunomodulator.

Although the effects of DC-SIGN targeting on immune responses have been well described for several pathogens, the molecular mechanisms underlying immune modulation by DC-SIGN, as well as for many other C-type lectins, has remained elusive for years. Co-localization of DC-SIGN with TLR4 has been hypothesized to mediate the enhanced TLR4

signaling, as has been described previously for SIGNR1, a murine homologue of DC-SIGN [68]. However, results from several studies in the last years indicate that DC-SIGN induces signal transduction pathways by itself. For example, DC-SIGN activation by a specific antibody denoted MR-1 results in activation of extracellular signal-regulated kinase (ERK) [69]. Although stimulation with an antibody might not specifically mimic what happens during pathogen interaction, it does indicate that DC-SIGN is capable of inducing signal transduction pathways. A study by Hodges et al. demonstrated that triggering of DC-SIGN with HIV-1 or a specific antibody denoted H-200 activates the Rho guanine nucleotide exchange factor (GEF) leukemia-associated Rho GEF (LARG), which in turn mediates Rho-GTPase activity [70]. Moreover, microarray studies revealed that cross linking of DC-SIGN with H-200 specifically affected the gene expression profile of DCs, with hundreds of genes being induced or downregulated after ligation [70]. Hence, DC-SIGN appears to be a pathogen receptor that, independent from other receptors, induces signal transduction pathways to modulate immune responses. Nevertheless, the specific molecular mechanisms responsible for immunomodulation by DC-SIGN have not yet been elucidated.

Dectin-1

Dectin-1 is a C-type lectin that recognizes carbohydrates independent of calcium. In contrast to what its name suggests ('dendritic cell-associated C-type lectin 1'), dectin-1 expression is not restricted to DCs, but is expressed by many other cell types including macrophages, monocytes, neutrophils, B cells and eosinophils [71-73]. Dectin-1 specifically recognizes β(1-3) and/or β (1-6)-glucans [74]. How dectin-1 recognizes carbohydrates is still unclear, since the receptor lacks conserved residues in its CRD that have previously been implicated in carbohydrate binding by other receptors [32]. In addition to its carbohydrate ligands, dectin-1 also recognizes an endogenous T cell ligand, which is most likely a protein instead of a carbohydrate [75].

Besides the capacity to bind to T cells and its consequent proposed role as a co-stimulatory molecule [75, 76], dectin-1 is particularly well known to function as a PRR. Dectin-1 recognizes several fungal pathogens including *Candida* spp [77], *Pneumocystis* spp [78], *Saccharomyces* spp [77], *Coccidiodes* spp [79] and *Aspergillus* spp [80] via recognition of β-glucans expressed in the fungal cell walls. Dectin-1 is the main receptor of β-glucans on both DCs [40, 43] and macrophages [73, 81], underlining its importance in fungal recognition. In addition, recent reports indicate that dectin-1 also recognizes mycobacteria, although the mycobacterial ligand for dectin-1 still remains to be identified [82-84]. Pathogen recognition

via dectin-1 mediates a multitude of immunological processes including the induction of specific cytokines and chemokines, reactive oxygen production and phagocytosis of pathogens.

The intracellular signaling pathways underlying dectin-1-mediated immune responses have been subject of intense investigation. Dectin-1 contains two tyrosine motifs in its cytoplasmic tail [75], which is sometimes referred to as an immunoreceptor tyrosine-based activation motif (ITAM)-like motif. However, in contrast to ITAM-receptors, signaling via dectin-1 only requires phosphorylation of the membrane proximal (but not the membrane distal) tyrosine [43], which has been hypothesized to be mediated by Src family kinases after pathogen binding [43, 85]. Phosphorylation of the tyrosine mediates binding and activation of spleen tyrosine kinase (Syk) [43, 85]. Activation of Syk is essential for dectin-1-induced cytokine and chemokine production via activation of NF-κB and MAP kinases. Syk activation induces the assembly of a scaffold consisting of Card9, B cell lymphoma 10 (Bcl-10) and mucosa-associated lymphoid-tissue lymphoma-translocation gene 1 (Malt1) [86]. This Card9-Bcl-10-Malt1 scaffold subsequently couples dectin-1 to the NF-κB pathway [86], implying that dectin-1, in contrast to DC-SIGN, autonomously induces the expression of response genes, i.e. without co-stimulation of other PRRs such as TLRs. Importantly, this dectin-1-induced NF-κB activation on DCs is crucial for the differentiation of T_H17 cells, which is required for effective clearance of fungal pathogens [87].

In addition to cytokine induction, Syk also mediates dectin-1-induced reactive oxygen production in macrophages and partially mediates dectin-1-induced phagocytosis by DCs [85]. However, several other dectin-1-mediated functions are independent of Syk activation. Besides the autonomous induction of cytokines, dectin-1 also collaborates with TLRs for the induction of cytokines [40]. Remarkably, this cross-talk between dectin-1 and TLRs is independent of Syk [37, 43]. In addition, phagocytosis by dectin-1 is Syk-independent in macrophages [37, 85]. These findings indicate that dectin-1 induces other, thus far unidentified signaling pathways besides Syk signaling.

Thesis outline

DCs are crucial for the induction of tailored immune responses against pathogens. Via the induction of a specific cytokine profile, DCs polarize T helper cell responses towards T_H1, T_H2, T_H17 or T_{reg}, which is essential for efficient clearance of pathogens while limiting detrimental side-effects of excessive immune activation. This pathogen-specific cytokine profile results from intracellular signaling and cross-talk between various PRRs.

In recent years, C-type lectins have been identified to play a vital role in the induction of immune responses, either alone or in concert with other PRRs. However, the molecular mechanisms underlying C-type lectin-induced immune responses were still largely unknown. Therefore, the aim of this thesis was to unravel the intracellular signaling pathways of two important C-type lectins: DC-SIGN and dectin-1.

In **chapter 2**, the role of the mannose cap on the cell wall of mycobacteria, which is important for the interaction of mycobacteria with DC-SIGN, was studied. Previous studies using purified components have described that the mannose cap on mycobacteria is a crucial factor in mycobacterial virulence. Using capless mutant bacteria we show that the mannose cap does not dominate the interaction between whole mycobacteria and host. This indicates that DC-SIGN-induced immune modulation in response to mycobacteria is not only mediated by the mannose-cap, but also by other mannose-structures expressed by mycobacteria. **Chapter 3** describes the identification of the intracellular signaling pathway through which DC-SIGN modulates cytokine induction by TLRs. This pathway is activated by several classes of pathogens including mycobacteria, viruses and yeasts. Pivotal for DC-SIGN signaling is the activation of kinase Raf-1, which mediates acetylation of NF-κB and thereby modulates immune responses to these pathogens. **Chapter 4** describes the identification of the proteins involved in DC-SIGN signaling upstream of Raf-1. Binding of mannose-expressing pathogens such as mycobacteria and HIV-1 to DC-SIGN induces the formation of a 'signalosome', a large complex of signaling proteins that cooperate to induce the activation of Raf-1. In striking contrast to mannose-expressing pathogens, fucose-expressing pathogens such as *H. pylori* disassemble the Raf-1 signalosome to induce a Raf-1-independent signaling cascade resulting in a distinct cytokine profile. This demonstrates that DC-SIGN induces ligand-specific signaling pathways, resulting in pathogen-specific immune responses.

In **chapter 5** the crucial role of Raf-1 signaling in dectin-1-induced immune responses was unravelled. Previous studies have demonstrated that dectin-1 induces NF-κB activation via Syk and Card9. However, it has remained unclear how dectin-1 specifically couples pathogen recognition to the induction of T_H1 and T_H17 polarizing cytokines. We show that dectin-1 independent from Syk signaling also induces the Raf-1 pathway. The ultimate immune response induced by dectin-1 is the result of an intricate interaction of these two signaling cascades: the Syk axis activates NF-κB subunits p65, c-Rel and RelB, while the Raf-1 pathway phosphorylates p65, thereby repressing RelB and enhancing p65 activity. Only combined are these two pathways able to induce T_H1

and T_H17 responses. In addition, it was found that cross-talk of dectin-1 with TLRs is dependent on Raf-1 signaling. In **chapter 6** the molecular mechanisms of dectin-1-mediated phagocytosis were studied. Dectin-1 is a well-known phagocytic receptor, but the proteins involved in dectin-1-mediated phagocytosis were still largely unknown. We demonstrate that leukocyte specific protein (LSP)1 associates with dectin-1 and that LSP1 is essential for phagocytosis of both live and heat-killed *C. albicans* by dectin-1 on macrophages. Finally, an overview of signaling by DC-SIGN and dectin-1, the implications for signaling by other C-type lectins, and the potential role of C-type lectin signaling for therapeutic applications are discussed in **chapter 7**.

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