Chapter

7

Discussion

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In the last few years, C-type lectins have emerged as pattern-recognition receptors (PRRs) that play a crucial role in the induction of immune responses against pathogens. However, the intracellular signaling cascades underlying C-type lectin functions were largely unknown. This thesis describes the identification of signaling pathways of the C-type lectins DC-SIGN and dectin-1, which play a profound role in immunity against numerous pathogens. This chapter will first give an overview of the current knowledge of signaling through these receptors and discuss the consequences of these signaling pathways on the induction of immunity against pathogens. Subsequently, implications of these findings for signaling by other C-type lectins and the potential of using this in therapeutic settings are discussed.

DC-SIGN

DC-SIGN interacts with a plethora of pathogens, which affects the immune responses against these pathogens by the induction of intracellular signaling cascades. The specific signaling pathway induced by DC-SIGN, and thereby also its effect on the subsequent immune response, depends on the composition of the pathogenic ligand involved. In this aspect, DC-SIGN signaling can currently be categorized into three distinct categories: (1) mannose-expressing pathogens such as HIV-1 and *M. tuberculosis* (Raf-1-dependent signaling), (2) fucose-expressing pathogens such as *H. pylori* (Raf-1-independent signaling) and (3) Salp15 (Raf-1-dependent signaling, but different from mannose-expressing pathogens). In this paragraph the distinct signaling pathways of DC-SIGN will be discussed in separate sections. First, DC-SIGN signaling through mannose-bearing pathogens will be discussed. Pivotal for DC-SIGN signaling by mannose-structures is the activation of kinase Raf-1. For clarity, first signaling 'upstream' Raf-1 activation will be discussed; subsequently, an overview of DC-SIGN signaling 'downstream' of Raf-1 and how this leads to modulation of immune responses will be given. Next, DC-SIGN signaling by other ligands such as fucose-expressing pathogens and the protein Salp15 is discussed in separate sections. Finally, the effects of DC-SIGN signaling on immune responses against pathogens are discussed, with main emphasis on the question if DC-SIGN signaling is beneficial for host or pathogen.

Mannose-expressing pathogens: signaling 'upstream'

The list of mannose-expressing pathogens that interact with DC-

SIGN is extensive and includes mycobacteria such as *M. tuberculosis* [1, 2] and *M. leprae* [3], viruses such as HIV-1 [4] and MV [5], and yeasts such as *C. albicans* [6] and *S. cerevisiae* [7]. In the last few years considerable progress has been made in unraveling DC-SIGN signaling via these mannose-bearing pathogens. Although most studies have focused on DC-SIGN signaling induced by the pathogens HIV-1 and *M. tuberculosis*, it seems likely that most of the mannose-expressing pathogens that interact with DC-SIGN induce the same signal transduction pathways [8]. Pivotal for DC-SIGN signaling by these mannose-expressing pathogens is activation of kinase Raf-1. The following section describes the activation of Raf-1 after pathogen binding to DC-SIGN.

How binding of mannose-structures to DC-SIGN leads to the initiation of signaling is still not completely clear. Binding of multivalent carbohydrate structures could induce clustering of DC-SIGN, which would allow the binding of adaptor-molecules that bridge the cytoplasmic tails of two or more DC-SIGN molecules and thereby initiate signaling, as has been hypothesized for dectin-1 [9]. However, absolute requirement for DC-SIGN clustering seems unlikely, since single mannose residues, which do not induce DC-SIGN clustering, are also able to induce Raf-1 activation after binding (Chapter 4). Our data show that initiation of DC-SIGN signaling is induced by the DC-SIGN signalosome, a protein complex associated with the cytoplasmic tail of DC-SIGN that is crucial for the activation of Raf-1.

The main scaffolding protein for the DC-SIGN signalosome is LSP1, an F-actin binding protein that associates with the cytoplasmic tail of DC-SIGN [10]. A study using DC-SIGN with truncated cytoplasmic tails indicated that specific regions in the cytoplasmic tail of DC-SIGN, which include the tyrosine and dileucine motifs, are required for binding [10]. However, our findings indicate that the tyrosine motif itself is not required for the association of LSP1 with the cytoplasmic tail of DC-SIGN, since LSP1 (Chapter 4), but not the tyrosine motif [8], is indispensable for Raf-1 activation.

In unstimulated conditions, LSP1 is associated with DC-SIGN together with the scaffolding proteins proteins kinase suppressor of Ras 1 (KSR1), connector enhancer of KSR 1 (CNK1) and Raf-1 itself. Upon pathogen binding, this DC-SIGN signalosome induces the activation Raf-1 via three routes (Figure 1). First, DC-SIGN mediates the activation of Ras, which is a prerequisite for Raf-1 activation by inducing a conformational change in Raf-1. Ras is a small G protein that mediates signaling by binding and hydrolysing GTP; the GTP-bound form of Ras is active and capable of binding Raf-1, whereas the GDP-bound form is inactive [11]. However, Ras itself also needs to be activated, which is mediated via GEFs. In DC-SIGN

signaling LARG is the GEF that activates Ras (Chapter 4). Thus, upon binding of mannose-bearing pathogens to DC-SIGN, LARG is recruited to the DC-SIGN signalosome where it can activate Ras, which subsequently mediates conformational changes of Raf-1.

However, activation of Ras alone is insufficient to activate the kinase activity of Raf-1 and additional phosphorylation of Raf-1 at at least two residues is required. Several phosphorylation sites on Raf-1 have been identified that are involved in its kinase activity [11]. Two of these phosphorylation sites are tyrosines 340/341 (Tyr340/341) and serine 338 (Ser338) [11]. Phosphorylation of Tyr340/341 on Raf-1 in response to DC-SIGN signaling is carried out by a member of the Src family of tyrosine kinases [8], most likely Lyn, Hck or Fgr, which are the most abundant Src kinases in DCs [12]. Although the mechanism is not completely clear, Src might be activated via CNK1, which has been shown to mediate Raf-activation by activating Src kinases [13]. Raf-1 phosphorylation on Ser338, the other residue crucial for Raf-1 activation, is induced by p21-activated kinases (Paks) [8, 11]. Pak activation is mediated by LARG-dependent activation of RhoA (Chapter 4), although it is currently unknown how RhoA is able to mediate Pak activation. Remarkably, LARG mediates a double function in the activation of Raf-1: first, it activates Ras that mediates conformational changes of Raf-1; and second, it activates RhoA that induces the phosphorylation of Raf-1 at Ser338.

In conclusion, our findings demonstrate the following model of DC-SIGN signaling upstream of Raf-1 (Figure 1): binding of mannose-expressing pathogens to DC-SIGN induces intracellular signals via the DC-SIGN signalosome that activates three routes that converge to activate Raf-1. First, pathogen binding induces the recruitment of LARG, which activates Ras and thereby induces conformational changes of Raf-1. In addition, LARG activates RhoA-dependent activation of Paks, which results in phosphorylation of Raf-1 at Ser338. Moreover, DC-SIGN activates Src kinases that induce the phosphorylation of Raf-1 at residue Tyr340/341. Combined, these pathways mediate the activation of Raf-1, which is essential for DC-SIGN-induced modulation of immune responses, as will be described in the next paragraph.

Mannose expressing pathogens: signaling 'downstream'

As described above, binding of mannose-expressing pathogens to DC-SIGN modulates immune responses to these pathogens by activation of Raf-1. This immune modulation is mediated by Raf-1-dependent targeting of transcription factor NF-κB. NF-κB activation is crucial for the induction of DC-mediated immune responses [14] and is activated by

several classes of PRRs after pathogen recognition. Although pathogen-binding to DC-SIGN does not activate NF-κB, the majority of pathogens that interact with DC-SIGN also interact with other PRRs that are capable of inducing NF-κB activation. For example, mycobacteria such as *M. tuberculosis* activate NF-κB through TLR2 and TLR4, while viruses such

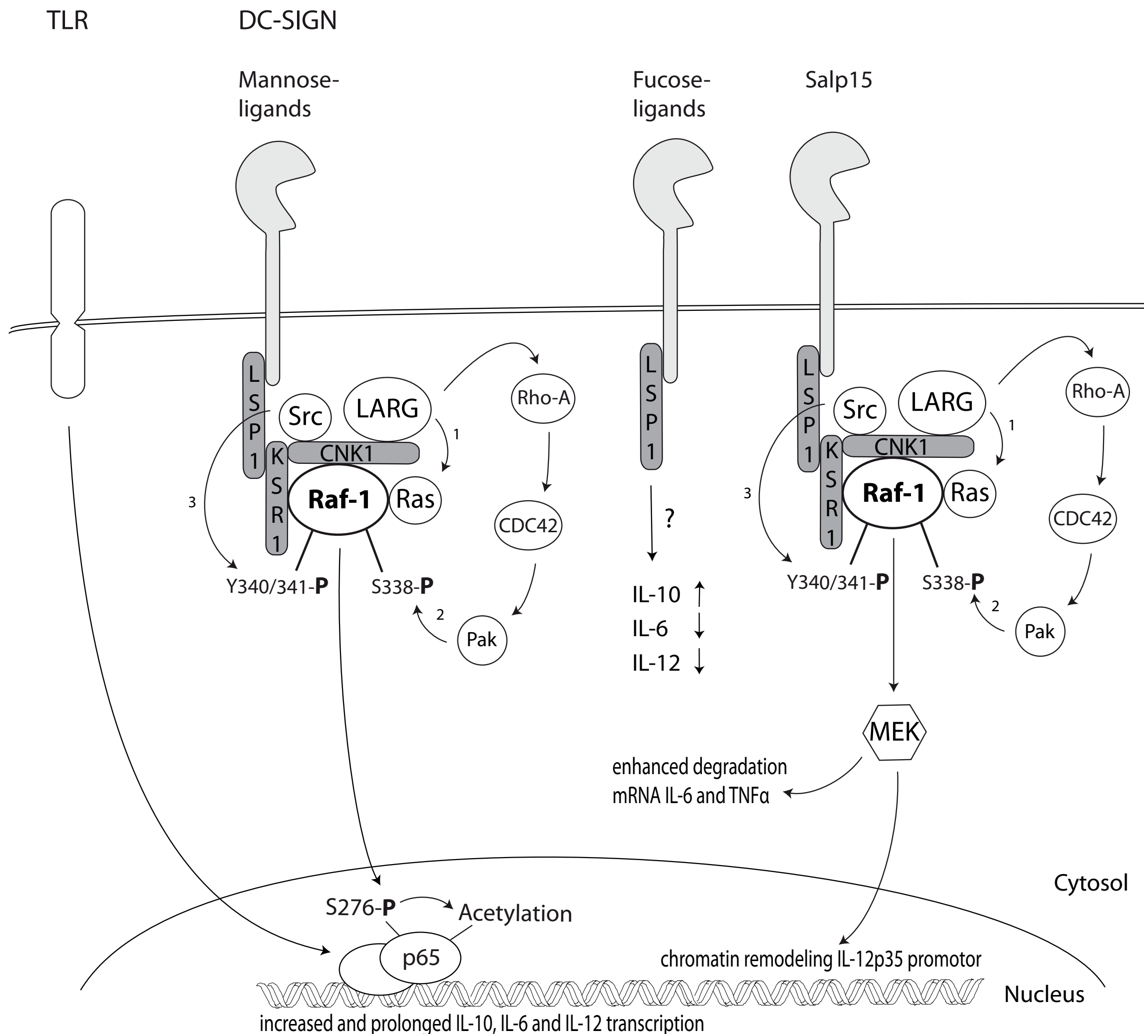


Figure 1 DC-SIGN signaling
DC-SIGN signaling is ligand-specific. (1) Binding of mannose-structures such as HIV-1 and *M. tuberculosis* induce signaling via a signalosome. When unstimulated, the DC-SIGN signalosome consists of scaffolding proteins LSP1, KSR1 and CNK1, which are associated with Raf-1. Binding of mannose-ligands induces three routes that converge to activate Raf-1. Recruitment of LARG activates Ras, which induces conformational changes of Raf-1 (route 1). In addition, LARG activates RhoA-dependent activation of Paks, which phosphorylates Raf-1 at Ser338 (route 2). Moreover, DC-SIGN activates Src kinases that induce the phosphorylation of Raf-1 Tyr340/341 (route 3). Subsequently, activated Raf-1 induces phosphorylation and acetylation of p65, which increases the transcription of IL-10, IL-6 and IL-12. (2) Signaling by fucose-structures such as *H. pylori* requires LSP1, but disengages the other components of the signalosome. Fucose-induced signaling enhances IL-10, but decreases IL-6 and IL-12 production via yet unknown mechanism. (3) Salivary tick protein Salp15 induces Raf-1 activation similar to mannose-structures, which in contrast to mannose-structures leads to MEK activation. Consequently, MEK activation impairs cytokine production by enhancing IL-6 and TNFα mRNA degradation and impairing chromatin remodeling at the IL12p35 promoter.

as HIV-1 induce NF-κB activation via endosomal TLR7 and TLR8. NF-κB comprises several different subunits, but TLR triggering predominantly leads to transactivation of heterodimers of NF-κB subunits p65 and p50 [8], of which p65 is the transcriptionally active component. In unstimulated cells, p65 is inactive and resides primarily in the cytosol in complex with any of the inhibitory IκB proteins. Upon stimulation, the IκB proteins are degraded and the p65-p50 dimers translocate into the nucleus where they bind to target DNA sequences, which promotes the transcription of numerous target genes including cytokines and chemokines.

The activity of p65 is regulated by several post-translational modifications such as phosphorylation and acetylation [15]. Strikingly, DC-SIGN-induced Raf-1 activation controls p65 activity by phosphorylation of p65 at serine 276 (Ser276) [8]. Subsequently, phosphorylation of p65 at Ser276 leads to acetylation of p65 [8, 16]. Acetylation of p65 can be mediated by two histone acetyltransferases (HATs), CREB binding protein (CBP) and p300, which in human DCs show high constitutive activity (unpublished data). Acetylation is known to have major consequences for p65 activity [17-19]. DC-SIGN-mediated acetylation of p65 leads to both increased and prolonged gene transcription, resulting in a strongly increased production of cytokines such as IL-10, IL-6 and IL-12 by DCs ([8] and Chapter 4).

Crucial for the understanding of DC-SIGN-signaling and its effect on immune responses is that DC-SIGN cannot activate p65 by itself, but modulates p65-activity when activation of p65 has been induced by another receptor. The induction of DC-SIGN-signaling by the mycobacterial component ManLAM does not induce activation of p65, and therefore does not induce any cytokine responses. However, upon stimulation with whole mycobacteria, TLR2 and TLR4 triggering induce the activation and translocation of p65 to the nucleus, which allows for subsequent phosphorylation and acetylation of p65 by DC-SIGN signaling (Figure 1).

A question that remains unanswered is the identity of the kinase responsible for phosphorylation of Ser276 of p65 upon DC-SIGN triggering. Nuclear translocation of p65 is required for phosphorylation of Ser276, indicating that phosphorylation is mediated by a nuclear kinase (unpublished data). A number of kinases have previously been reported to phosphorylate Ser276 on p65, such as mitogen- and stress-activated kinase-1 (MSK1) [20], but inhibitors against this kinase do not inhibit ManLAM-induced IL-10 upregulation [8]. Hence, the identity of this nuclear kinase is still under investigation.

Modulation of immune responses by DC-SIGN is probably not restricted to TLR-activation, but instead might also include other NF-κB dependent

responses. Hypothetically, DC-SIGN signaling modulates the induction of any immune response that is p65-dependent, since phosphorylation and acetylation of p65 drastically alter the transcription rate and the duration of p65 activation. Moreover, DC-SIGN signaling could also affect immune responses of PRRs that induce other transcription factors such as RelB, which dimerizes with p65 and becomes inactivated after DC-SIGN-induced phosphorylation of p65 at Ser276 [21], as is the case during dectin-1 signaling (which is described later on in this Chapter). Thus, since immune activation of DCs by mannose-expressing pathogens critically depends on NF-κB activation, DC-SIGN signaling might play a crucial role in directing immune responses to numerous pathogens.

DC-SIGN signaling by fucose-expressing pathogens

In contrast to DC-SIGN signaling by mannose-expressing pathogens, very little is known about signaling by fucose-expressing pathogens. Most of the knowledge on DC-SIGN signaling by fucose-expressing pathogens is based on studies using phase-variants of *H. pylori* and soluble egg antigens of the parasite Schistosoma mansoni. The human gastric pathogen *H. pylori* spontaneously switches LPS Lewis antigens on and off, a process known as phase-variable expression, which results in LewisY(LeY)+ and LeY- bacteria within a single strain [22]. Both variants interact with PRRs such as TLRs and thereby induce DC-induced immune activation, but only the LeY+ strain interacts with DC-SIGN. DC-SIGN signaling induced by LeY+ H. pylori drastically alters the cytokine profile compared to the LeY- strain, with increased production of anti-inflammatory IL-10 and decreased production of pro-inflammatory cytokines IL-6 and IL-12 (Chapter 4). Remarkably, this cytokine profile is strikingly distinct from mannose-expressing pathogens, whose binding to DC-SIGN increases both anti-inflammatory IL-10 and pro-inflammatory IL-6 and IL-12. As suggested by their differences in cytokine profile, mannose- and fucose-structures induce different signaling pathways (Figure 1). Although both mannose- and fucose-induced DC-SIGN signaling requires LSP1, fucose-structures do not signal via the DC-SIGN signalosome. Instead, binding of Lewis-structures to DC-SIGN disassembles the signalosome: KSR1, CNK1 and Raf-1, which in unstimulated condition are associated with DC-SIGN, are disengaged from cytoplasmic tail of DC-SIGN after Lewis-binding and are not required for subsequent signaling.

The mechanisms responsible for the fucose-specific cytokine profile are still unclear. Fucose-signaling most likely does not involve p65 phosphorylation at Ser276 and p65 acetylation, since acetylation inhibitors do not inhibit Lewis-induced cytokine induction (unpublished data). Possibly, fucose structures induce other post-translational modifications

of NF-κB. In addition, attenuated cytokine production might not be regulated at the level of transcription, but instead be orchestrated by other mechanisms such as mRNA degradation. Additional investigations are required to unravel this novel DC-SIGN signaling pathway.

DC-SIGN signaling by Salp15

In addition to mannose and fucose ligands, a third category of DC-SIGN signaling is induced by the protein Salp15. Salp15 is an immunomodulatory protein produced by the salivary glands of Ixodes scapularis ticks [23]. The presence of immunosuppressive molecules such as Salp15 in tick saliva facilitate tick feeding, but also greatly enhance the tick's ability to transmit human pathogens such as *Borrelia burgdorferi* [24], the causative agent of Lyme disease.

B. burgdorferi activates DCs by interaction with TLRs [25, 26]. However, binding of Salp15 to DC-SIGN on DCs inhibits TLR2- and TLR4-induced production of pro-inflammatory cytokines IL-12, IL-6 and TNFα [27]. Similar to DC-SIGN signaling by mannose-expressing pathogens, modulation of TLR-responses by Salp15 is completely dependent on the activation of Raf-1 [27]. Moreover, signaling upstream of Raf-1 is most likely mediated via the DC-SIGN signalosome, since silencing of any of the signalosome components completely abrogates Salp15-induced immune modulation (unpublished data). However, in contrast to ManLAM, Salp15-induced signaling does not lead to phosphorylation or acetylation of p65, but instead leads to activation of mitogen-activated protein kinase kinase (MEK). Although MEK is a well-characterized upstream effector of ERK, remarkably Salp15-induced activation of MEK does not lead to ERK activation [27]. Alternatively, Salp15-induced Raf-1/MEK signaling regulates the inhibition of pro-inflammatory cytokines at different levels: inhibition of IL-6 and TNFα is caused by enhanced degradation of their respective mRNAs, while decreased production of IL-12 results from impaired nucleosome remodeling at the IL12p35 promotor (Figure 1) [27].

The reason behind the different downstream effectors of Raf-1 after activation by Salp15 compared to mannose-structures is still unclear. One explanation could be that Salp15 also activates other receptors, which modulate DC-SIGN signaling. Besides DC-SIGN, Salp15 has also been described to bind to CD4 [28]. Although no direct physical association has been found between both proteins [29], DC-SIGN and CD4 have been found to colocalize on the cell-surface [30]. On T cells, binding of Salp15 to CD4 modulates actin polymerization [28]. A central theme in signaling is bringing together kinases and substrates, and impairment of actin polymerization might bring Raf-1 together with a different downstream

effector compared to ManLAM-induced DC-SIGN-signaling. Therefore, co-ligation of DC-SIGN and CD4 by Salp15 might be responsible for the induction of this distinct signaling pathway by affecting the subcellular localization of either Raf-1 or its downstream effectors. Additionally, future research on the molecular mechanism of Salp15-induced DC immunosupression is required to elucidate how MEK affects mRNA stability and nucleosome remodeling. Although MEK kinases are well known to activate ERK 1 and 2, Salp15 does not signal through ERK kinases [27]. The downstream effectors of MEK are most likely involved in post-translational modifications of the proteins involved in mRNA decay and nucleosome remodelling.

Thus, although Salp15 binding to DC-SIGN does induce Raf-1 activation similar to mannose-expressing pathogens, the downstream effectors that modulate TLR-induced activation are different. Although Raf-1 plays a central role in both mannose- and Salp15-induced DC-SIGN signaling, the downstream effectors of Raf-1 regulate the subsequent modulation of cytokine responses.

DC-SIGN signaling: beneficial for host or pathogen?

DC-SIGN signaling by pathogens does not modulate immune responses uniformly, but instead immune modulation depends on the pathogen involved. The reason for this pathogen-specific immune modulation by DC-SIGN is two-fold. First, every pathogen triggers a specific set of PRRs in addition to DC-SIGN. Since different PRRs induce different signaling cascades, the ultimate effect of DC-SIGN signaling on immune responses depends on cross-talk of DC-SIGN with the signaling cascades of the specific set of PRRs involved. In addition, as described above, DC-SIGN itself induces different signaling pathways depending on the DC-SIGN ligand involved (i.e. mannose-ligands, fucose-ligands, or Salp15), thereby generating pathogen-specific modulation of cytokine induction. However, a pivotal question that remains is whether DC-SIGN-induced modulation of immune responses is beneficial for the host or whether DC-SIGN signaling is induced by pathogens to subvert an effective immune response and promote their growth and survival. Although for years the idea that pathogens target DC-SIGN to promote their own survival has been the prevailing notion [1, 31], it is gradually becoming clear that DC-SIGN-mediated immune responses are not always disadvantageous for the host. Whether DC-SIGN signaling favours host or pathogen seems to be dependent on the pathogen involved. Therefore, in the following section the consequences of DC-SIGN signaling for host and pathogen are discussed for a selection of the different DC-SIGN-interacting pathogens individually.

In the case of HIV-1 (a mannose-expressing pathogen), DC-SIGN signaling appears to be beneficial for the pathogen. First of all, DC-SIGN plays an important role in HIV-1 infection of the host by mediating the transmission of viruses from DCs to T cells [4]. DCs capture HIV-1 through the high-affinity interaction of DC-SIGN with gp120, and subsequently mediate transmission of the virus to T cells through the formation of a so-called infectious synapse between HIV-1-infected DCs and CD4+ T cells [32]. Recently, Hodges et al. demonstrated that DC-SIGN-signaling is responsible for the formation of this viral synapse [33]. In addition, DC-SIGN signaling suppresses effective immune responses against HIV-1 through several mechanisms. DC-SIGN signaling enhances TLR-induced IL-10 production [8] and impairs TLR-induced dendrite formation of DCs and T cell proliferation [33]. Moreover, HIV-1-induced DC-SIGN signaling mediates effects independent of TLR activation. Microarray studies revealed that HIV-1-binding specifically affected the gene expression profile of DCs, with hundreds of genes being induced or downregulated after ligation [33]. One of the most notable features of the DC-SIGN signaling program is the induction of ATF3, a negative regulator of TLR4 [34], suggesting that DC-SIGN signaling in addition to modulation might also repress TLR4 signaling. Thus, by mediating the transmission of HIV-1 to T cells and impairing DC maturation and T cell proliferation, DC-SIGN signaling strongly promotes systemic infection of the host.

For mycobacteria (mannose-expressing pathogens) DC-SIGN signaling was initially thought to be a mechanism through which DC function was suppressed to promote pathogen survival, based on impaired DC maturation and enhanced production of the immunosuppressive cytokine IL-10 by DCs after stimulation with ManLAM [1]. However, besides upregulation of the immunosuppressive cytokine IL-10, DC-SIGN signaling by mycobacteria also upregulates the production of several pro-inflammatory cytokines such as IL-12 and IL-6 (Chapter 4). This suggests that DC-SIGN signaling modulates rather than suppresses the immune response. A T_H1 response is critical for effective protection against mycobacteria [35, 36], although recently also T_H17 responses have been described to be involved [37]. However, a strong long-lasting T_H1 response might also detrimental for the host by enhancing tuberculosis-induced pathology. Although additional investigations are required to determine how DC-SIGN signaling modulates the polarization of T helper responses, DC-SIGN seems to have a profound effect on the pathogenesis during mycobacterial infection. In this regard, genetic epidemiology has yielded conflicting data. One study suggested that a single nucleotide polymorphism (SNP) that leads to decreased DC-SIGN expression is associated with reduced risk for cavitory tuberculosis

disease [38]; in contrast, another study demonstrated that SNPs in the DC-SIGN promoter region that lead to increased DC-SIGN expression appear to be protective against tuberculosis [39]. In addition, in a recent study it was reported that transgenic mice expressing human DC-SIGN actually show enhanced clearance of mycobacteria [40]. DCs from DC-SIGN-mice displayed reduced tissue damage and prolonged survival. These findings suggest that instead of favoring the immune evasion of bacteria, DC-SIGN signaling might promote protection by limiting tuberculosis-induced pathology.

For *H. pylori* (a fucose-expressing pathogen), the situation is not completely clear-cut. *H. pylori* infects almost half of the population worldwide and represents the primary cause of gastritis [41]. However, only a limited number of patients with *H. pylori*-induced gastritis develop chronic and life-threatening complications such as peptic ulcers. The appropriate T helper response required for limiting complications is still a matter of debate. A predominant T_H2 response is not beneficial for the host since it does not mediate protection, but on the other hand a dominant T_H1-profile is associated with increased gastric inflammation and disease [42]. Therefore, it has been proposed that immunity against *H. pylori*-induced complications requires a mixed T_H1/ T_H2 response that generates protection without disproportionate immunopathology [42]. If this hypothesis holds true, DC-SIGN signaling might benefit the host, since LeY-positive variants enhance IL-10, attenuate T_H1-polarizing IL-12 (Chapter 4), and direct a dominant T_H1 response to a mixed T_H1/ T_H2-profile [43]. However, the genetic composition of the host may also play an important role in successful colonization by *H. pylori* [44]. In the genetically very diverse human population individuals differ in their capacity to induce T_H1 and T_H2 polarizing cytokines. Since *H. pylori* can spontaneously switch between phase-variants, the host may positively select the phase-variant that displays the ideal composition for colonization of that particular host, resulting in selective outgrowth of one variant. If this indeed occurs, DC-SIGN signaling induced by LeY-positive variants might be a mechanism to promote persistent colonization of *H. pylori*, thereby favouring the pathogen.

The presence of Salp15 in tick saliva suppresses immune responses to facilitate tick feeding. However, Salp15 also affects the immune response against *B. burgdorferi* [24], which can be transmitted to the host during tick feeding. Binding of Salp15 to DC-SIGN on DCs attenuates the expression of pro-inflammatory cytokines and impairs T cell proliferation [27], supporting the hypothesis of immune suppression by DC-SIGN after Salp15-stimulation. In addition, effective protection against *B. burgdorferi* requires the initial induction of a T_H1 response [45-47]. However, DC-SIGN

signaling by Salp15 attenuates the production of the T_H1-polarizing cytokine IL-12 [27] and skews T helper cell responses towards T_H2 (MAWP de Jong, personal communications). These findings indicate that DC-SIGN signaling by Salp15 prevents a protective immune response against B. burgdorferi and is therefore specifically beneficial for the pathogen.

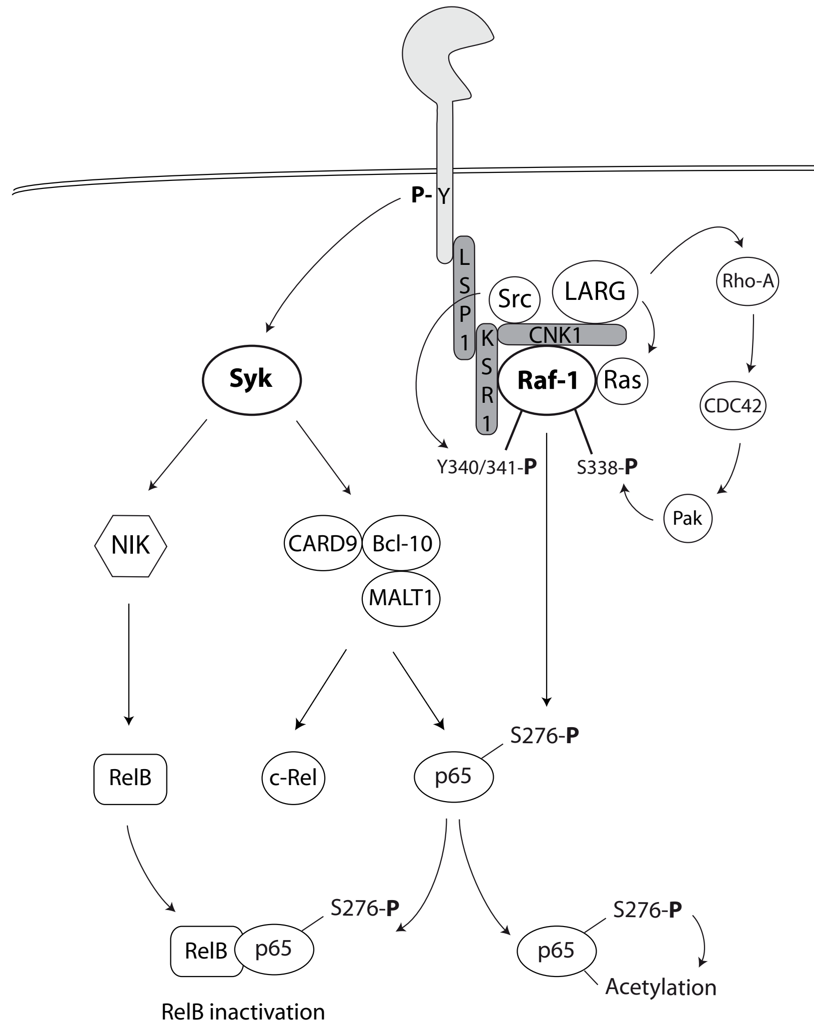
Remarkably, most DC-SIGN-binding pathogens are microbes that in general induce latent infections, such as *M. tuberculosis*, *M. leprae*, *C. albicans* and *H. pylori*. This suggests that modulation of the immune response via interaction with DC-SIGN results in a 'steady-state' that attenuates immune-activation in order to limit detrimental side effects of a full-blown immune response, but in turn also allows for pathogen survival.

Dectin-1

Dectin-1 plays a key role in immunity against several fungal pathogens through the recognition of β-glucans [48-51], but has recently also been identified to mediate immune responses against mycobacteria via interaction with a yet unidentified ligand [52-54]. Dectin-1 simultaneously induces at least two independent pathways that underlie dectin-1-mediated immune responses. Since β-glucan is the only dectin-1 ligand identified thus far, dectin-1 has not been shown to induce ligand-specific signaling such as DC-SIGN, although the downstream effects of dectin-1 signaling might be cell-type dependent. Innate signaling by dectin-1 mediates several immunological processes, including the autonomous induction of cytokines, modulation of TLR-induced cytokines, and phagocytosis. In the following sections, the signaling pathways underlying these different dectin-1-mediated functions will be discussed individually.

Cytokine induction and T helper cell differentiation

Cytokine induction by dectin-1 is mediated by an intricate interaction of two distinct signaling pathways. These two signaling pathways are induced independently, but cooperate to induce a specific cytokine program. One of the pathways activated by dectin-1 after pathogen binding is mediated by the kinase Syk (Figure 2A). Syk signaling is initiated by phosphorylation of the membrane proximal tyrosine motif in the cytoplasmic tail of dectin-1, possibly by Src kinases, resulting in association and activation of Syk [9, 55]. Subsequently, Syk activation induces the assembly of a scaffold consisting of Card9, Bcl-10 and Malt1 [56]. This



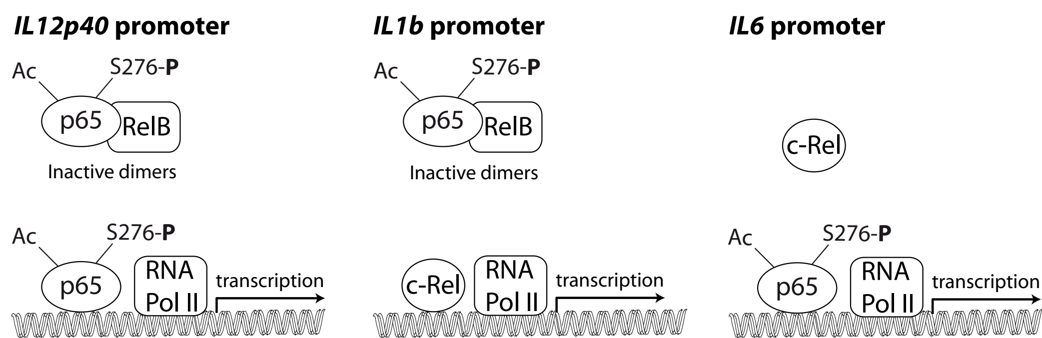


Figure 2 Dectin-1 signaling
Dectin-1 induces two independent signaling pathways that cooperate to induce a specific cytokine profile. **A**. Dectin-1 induces both Syk and Raf-1 signaling. Syk signaling activates NF-κB subunits p65 and c-Rel as well as NIK-dependent RelB. Both p65 and RelB activity are controlled by the second signaling pathway via Raf-1. Raf-1 activation via the signalosome leads to phosphorylation of p65 at Ser276, which has distinct effects on NF-κB activation: phosphorylated p65 represses active RelB by forming inactive p65-RelB dimers, whereas acetylation of p65 increases its DNA binding activity and transcriptional activity. **B**. Induction of transcription by NF-κB subunits at *IL12p40*, *IL1b* and *IL6* promoter after dectin-1 stimulation. Phosphorylated p65 represses active RelB by forming inactive p65-RelB dimers, which allows IL-12p40 and IL-1β expression, whereas acetylation of p65 increases its DNA binding activity, which enhances *IL12p40* and *IL6* transcription. Consequently, combined signaling of Syk and Raf-1 by dectin-1 triggering leads to the production of T_H1 (IL-12p70) and T_H17 (IL-1β, IL-6 and IL-23) polarizing cytokines.

Card9-Bcl-10-Malt1 scaffold subsequently couples dectin-1 activation to the NF-κB pathway [56]. Therefore dectin-1, in contrast to DC-SIGN, is able to induce cytokine production on its own and does require co-stimulation of TLRs. Remarkably, we showed that Syk signaling activates not only canonical NF-κB proteins p65 and c-Rel, but also the non-canonical NF-κB protein RelB [57].

In addition to Syk, dectin-1 induces a second signaling pathway mediated by the kinase Raf-1 (Figure 2A). Importantly, Raf-1 activation is not induced by the Syk signaling pathway, indicating that dectin-1 induces two independent signaling pathways, one through Syk and one through Raf-1. How binding of pathogens to dectin-1 initiates Raf-1 signaling is not yet completely clear. Similar to DC-SIGN, Raf-1 activation by dectin-1 requires the protein LSP1 (unpublished data). LSP1 directly associates with the cytoplasmic tail of dectin-1 (Chapter 6), although the motif in the cytoplasmic tail of dectin-1 responsible for LSP1-binding is still unclear. Moreover, Raf-1 activation by dectin-1 is most likely mediated via a complex of signaling proteins reminiscent of the DC-SIGN-signalosome, since silencing of any of the DC-SIGN signalosome components completely abrogates Raf-1-induced immune responses by dectin-1 (unpublished data). This implies that dectin-1 utilizes the same upstream machinery as DC-SIGN for the activation of Raf-1 (Figure 2A): first, pathogen binding induces the recruitment of LARG, which activates Ras and thereby induces conformational changes of Raf-1; in addition, Raf-1 is phosphorylated at Ser338 by LARG-RhoA-Pak dependent signaling and phosphorylated at Tyr340/341 by Src kinases.

Although upstream the two dectin-1 pathways, Syk and Raf-1, operate independently, downstream the two pathways ‘integrate’ at the level of NF-κB activation (Figure 2A). Syk signaling leads to the activation of NF-κB subunits p65, c-Rel, and RelB. On the other hand, Raf-1 signaling induces the phosphorylation of p65 at Ser276. This Raf-1-induced phosphorylation of p65 at Ser276 regulates NF-κB activity at two distinct levels. First, p65 phosphorylation at Ser276 induces the formation of inactive p65-RelB dimers [57]. Although a previous study reported that the formation p65-RelB dimers represses both RelB and p65 activity [21], our data demonstrate that only RelB is inactivated after p65-RelB dimerization [57]. Second, phosphorylation of p65 at Ser276 leads to acetylation of p65 by the activity of HATs [16, 57], as described previously for DC-SIGN [8]. Acetylation of p65 has major effects on its transcriptional activity by enhancing the transcription rate and the duration of p65 activation [17-19]. Combined, the two consequences of Raf-1-induced phosphorylation of p65 at Ser276, i.e. RelB inactivation and p65 acetylation, drastically affect Syk-induced cytokine expression.

Pathogen recognition by dectin-1 leads to the induction of T_H1 and T_H17 responses, which are both vital for effective immunity against fungal pathogens [49]. Strikingly, the integration of Syk and Raf-1 signaling is crucial for the induction of these specific T helper responses through the induction of T_H1- and T_H17-polarizing cytokine profiles. A key factor for T_H1 differentiation is IL-12, which is a dimer composed of subunits IL-12p40 and IL-12p35. Transcription of both IL-12 subunits is regulated differently. Transcription of *IL12p40* is regulated by binding of NF-κB subunits p65 and RelB to the *IL12p40* promoter (Figure 2B). The Syk pathway activates both p65 and RelB, but since RelB displays a greater affinity predominantly RelB will be associated with the *IL12p40* promoter after Syk signaling alone. Binding of RelB to the *IL12p40* promoter prevents RNA polymerase II recruitment, resulting in severely repressed transcription of *IL12p40*. Thus, Syk signaling alone would only induce very little IL-12p40, and therefore very little functional IL-12. However, besides Syk signaling dectin-1 also induces Raf-1 signaling. As described above, Raf-1 activation phosphorylates Ser276 of p65, which leads to both inactivation of RelB through the formation of p65-RelB dimers, and acetylation of p65 that increases p65 transcriptional activity. As a result, inactivated RelB cannot associate with the *IL12p40* promoter and can therefore not repress transcription. Instead, p65 binds to the *IL12p40* promoter, resulting in recruitment of RNA polymerase II and enhanced transcriptional activity because of p65 acetylation. Although Raf-1 signaling also enhances transcription of *IL12p35*, albeit through a different mechanism [57], IL-12p40 is limiting for the production of bioactive IL-12 in this situation and thereby for the generation of T_H1 immunity. In addition, IL-12p40 is also required for the generation of bioactive IL-23, which is a heterodimer composed of IL-12p40 and IL-23p19. Although *IL23p19* transcription is attenuated by Raf-1 signaling [57], Raf-1-induced *IL12p40* transcription is limiting for the generation of bioactive IL-23, similar to the previously described production of bioactive IL-12. Hence, Raf-1 signaling is also crucial for the induction of the T_H17-polarizing cytokine IL-23.

Besides IL-23, human T_H17 responses are also promoted by the cytokines IL-1β and IL-6 [58-62]. Importantly, Raf-1 is crucial for transcription of both of these cytokines. Transcription of *IL1b* is regulated by binding of NF-κB subunits c-Rel and RelB to the *IL1b* promoter (Figure 2B). As described previously, Syk signaling activates both c-Rel and RelB, but similar to *IL12p40* RelB will primarily associate with the *IL1b* promoter when only the Syk pathway is activated, since RelB displays a greater affinity for the promoter than c-Rel. Remarkably, the *IL1b* gene in DCs is transcribed from a 'poised' promoter architecture [57], indicating that

RNA polymerase II is already recruited to the transcription initiation site of *IL1b* prior to stimulation. However, binding of RelB to the promoter dissociates RNA polymerase II from the *IL1b* promoter, thereby preventing *IL1b* transcription. Strikingly, Raf-1 signaling inactivates RelB, thereby allowing c-Rel to associate with the promoter, resulting in effective transcription of *IL1b*. As such, Raf-1 signaling is crucial for production of IL-1β, since IL1b transcription is completely impaired upon Syk signaling alone. For IL-6, Syk signaling induces both p65 and c-Rel that associate with the *IL6* promoter, but Raf-1 signaling induces the acetylation of p65, thereby increasing the binding of p65 to the promoter and enhancing transcriptional activity (Figure 2B). Hence, both Syk and Raf-1 signaling are required for induction of a T_H17 cytokine program; Syk signaling induces the activation of the different NF-κB subunits, while Raf-1 signaling modulates the activity of the NF-κB subunits to mediate transcription of the specific response genes. Although T helper assays clearly demonstrate the requirement of both Syk and Raf-1 signaling for actual induction of T_H17 responses (unpublished results), additional investigations are required to assess how the different signaling pathways induce production of TGFβ and IL-21, two cytokines that have recently been identified to be crucial for T_H17 induction from naïve T cells in humans [59, 62].

When recapitulating the findings of dectin-1 signaling, the activation of two partly antagonistic pathways appears paradoxically at first sight: dectin-1 induces Syk signaling to activate RelB, while on the other hand dectin-1 induces Raf-1 signaling to inactivate RelB (Figure 2A). Presumably, RelB activation somehow plays a role in dectin-1-induced immune responses. Importantly, Raf-1 signaling does not completely impair Syk-induced RelB activation [57], leaving a small amount of active RelB. While RelB can suppress the transcription of certain cytokines such as *IL12p40* and *IL1b*, this small amount of RelB only moderately suppresses *IL12p40* transcription and does not affect *IL1b* transcription. However, instead of repressing transcription, RelB can also induce transcription of response genes, such as the chemokines *CCL17* and *CCL22*. Notably, the small proportion of active RelB is sufficient to mediate the production of CCL17 and CCL22 [57]. Thus, the simultaneous activation and partial inactivation of RelB appears to be a mechanism that induces T_H1 and T_H17 cytokines, but also allows a moderate expression of T_H2 chemokines such as CCL17 and CCL22.

Cross-talk with TLRs

As described above, dectin-1 autonomously induces the production of cytokines that direct T helper cell polarization. However, DC-pathogen

interactions mostly involve recognition through more than one PRRs, resulting in crosstalk between different PRRs that determines the ultimate expression of cytokines. Notably, dectin-1 modulates TLR-induced expression of cytokines [9, 63]. Since dectin-1 and TLR2 seem to co-localize after binding of common ligands ligands such as zymosan [64], it has been suggested that dectin-1 and TLRs might form a supramolecular signaling complex [65], which might also include tetraspanins such as CD63 [66] and CD37 [67]. This integration of dectin-1 signaling with TLR activation might involve kinase recruitment to dectin-1 and subsequent TLR phosphorylation as described for TLR2 [68], which could explain the requirement of dectin-1 for TLR2-induced cytokine production after stimulation with mycobacteria [54]. However, our data indicate that cross-talk between dectin-1 and TLRs is mediated by both Raf-1 and Syk signaling by dectin-1 [57]. TLR triggering induces the activation of p65, which mediates immune activation by the expression of numerous cytokines. Raf-1 signaling by dectin-1 mediates the acetylation of p65, which increases and prolongs the transcriptional activity of p65 [17-19], thereby enhancing the production of the TLR-induced genes. In addition, as also described by others [69], Syk contributes to dectin-1-TLR cross-talk as well. Syk signaling by dectin-1 modulates TLR-induced gene transcription through the activation of NF-κB subunits c-Rel and RelB, which can affect the transcription of p65-regulated genes through e.g. competitive binding at the relevant promoter. Remarkably, the contribution of the Syk pathway in TLR–dectin-1 crosstalk is dependent on the TLR involved, since dectin-1-induced Syk signaling is more important for TLR2- than TLR4-mediated cytokines [57]. In conclusion, crosstalk between TLRs and dectin-1, and possibly other NF-κB-inducing PRRs and dectin-1, is mediated by both Raf-1 and Syk signaling.

Phagocytosis

Dectin-1 is one of the main receptors for phagocytosis of fungal pathogens [50, 65], but has also been found to mediate phagocytosis of mycobacteria [53]. The molecular mechanisms behind dectin-1-mediated phagocytosis are still largely undefined, but appear to be cell-type specific. In macrophages, LSP1 is a crucial mediator of phagocytosis by dectin-1 (Chapter 6), while phagocytosis is independent of Syk signaling [55, 70]. As described previously, LSP1 directly associates with the cytoplasmic tail of dectin-1. However, the signaling pathways responsible for dectin-1-mediated phagocytosis downstream of LSP1 are still ill defined. Phosphorylation of the membrane-proximal tyrosine motif in the cytoplasmic tail has been found to be essential for dectin-1-mediated uptake by murine macrophages [70], suggesting that phagocytosis through LSP1-induced signaling requires phosphorylation of this motif. As

described previously, LSP1 mediates dectin-1-signaling via the previously described signalosome that leads to Raf-1 activation. The Raf-1 signaling pathway comprises several proteins that have previously been found to be involved in induction of phagocytosis by dectin-1, such as the Rho GTPases Cdc42 and Rac1 [70], suggesting that dectin-1-mediated phagocytosis could be induced via the Raf-1 pathway. However, direct involvement of proteins of the Raf-1 signaling pathway in phagocytosis by dectin-1 has not yet been assessed. In addition, LSP1 has been described to be involved in signal transduction via interaction with several other signaling proteins[71-73], suggesting that LSP1 might also activate an other pathway for the induction of phagocytosis. Remarkably, LSP1 directly interacts with PKC [74], which previously has been shown to be required for dectin-1-mediated phagocytosis by macrophages [70].

As mentioned earlier, the molecular mechanisms of dectin-1 mediated phagocytosis appear to be cell-type specific. In contrast to macrophages, dectin-1-mediated phagocytosis by DCs is partly dependent on Syk [9], although the mechanism behind this discrepancy is unclear. In addition, dectin-1-mediated phagocytosis by microglia cells is Syk-independent [75]. Remarkably, microglia cells activate Syk upon dectin-1 stimulation, but this does not lead to the production of cytokines [75]. This illustrates that not only phagocytosis-, but also cytokine-inducing signaling pathways of dectin-1 are cell-type specific.

Taken together, several proteins required for dectin-1-mediated phagocytosis in macrophages have been identified, including LSP1, Rho GTPases and PKC, but an integrated model is still lacking and requires additional investigations.

Implications for other C-type lectins

In the last years, it has become clear that C-type lectins are not just involved in pathogen binding and uptake, but instead can function as genuine PRRs through the induction of intracellular signaling cascades. Since the signaling pathways of a limited number of C-type lectins have now been identified, it is tempting to speculate whether the knowledge acquired thus far has implications for signaling of other C-type lectins. Although not all C-type lectins might be capable of signaling autonomously, most C-type lectins harbor several motifs in their cytoplasmic tails that have been implicated in the recruitment of signaling proteins. The composition of the cytoplasmic domains varies greatly, both in length and in potential signaling motifs (see Introduction

Figure 3). However, our current understanding of C-type lectin signaling suggests that the different receptors do not all induce distinct signaling cascades, but instead that certain C-type lectins induce similar pathways. Syk signaling is induced by at least three different C-type lectins: dectin-1, CLEC-2 [76], and CLEC9A [77]. CLEC-2 is a C-type lectin expressed in myeloid cells [78] and platelets [76] that signals after binding of the snake venom rhodocytin, but is also involved in capturing HIV-1 by platelets [79]. CLEC9A expression (which has also been named DNGR1) is restricted to a small subset of blood DCs that express BDCA3, but is also expressed in other tissues [77, 80, 81]. The ligand(s) and the physiological function of CLEC9A are still largely unknown, but seem to involve the recognition of dead cells. The mechanism of Syk activation by these receptors seems to be identical: ligand binding induces Src kinase-induced phosphorylation of the YxxL motif in the cytoplasmic tail, which leads to Syk activation and subsequent downstream tyrosine phosphorylation events [9, 76, 77, 82]. The only other C-type that bears a YxxL motif is DC-SIGN, which does not signal via Syk [8]. This might be caused by the distinct series of amino acids that precede the YxxL motif of DC-SIGN (QTRG), which differs from dectin-1 and CLEC-2 (DEDG) [82]. Nevertheless, signaling via Syk does not necessarily require the expression of the YxxL motif by C-type lectin itself: macrophage-inducible C-type lectin (Mincle) induces Syk activation through association with the Fc receptor common γ-chain (FcRγ) [83], an ITAM-expressing adaptor molecule that can activate Syk. Coupling of Mincle to this adaptor molecule is mediated by the interaction of a positively charged residue in the cytoplasmic tail of Mincle with the negatively charged FcRγ. Several other C-type lectins, such as dectin-2 [84], BCDA-2 [85] and (murine) DCAR [86], express a similar positively charged residue, suggesting that these receptors might also couple to negatively charged adaptor molecules for the induction of downstream signaling. Thus, although the mechanism of activation might be different, several C-type lectins mediate their functions via activation of Syk.

In addition to Syk, Raf-1 signaling is induced by multiple C-type lectins as well: both DC-SIGN and dectin-1 induce Raf-1 signaling to direct immune responses to pathogens. In contrast to Syk signaling, the tyrosine motif does not appear to be required for DC-SIGN-induced activation of Raf-1 [8], leaving no shared motif for Raf-1 activation in the cytoplasmic tail of DC-SIGN and dectin-1. However, crucial for Raf-1 activation by both receptors is association of LSP1 with the cytoplasmic domains (Chapter 4 and unpublished data). Besides DC-SIGN and dectin-1, LSP1 has also been found to associate with the C-type lectins Langerin and L-SIGN [10]. Langerin is predominantly expressed on LCs present in the epidermis of the skin. Similar to DC-SIGN and dectin-1, Langerin

recognizes pathogens such as HIV-1 [87, 88], M. leprae [89] and C. albicans [90]. Although it is yet unclear whether Langerin plays a role in induction or modulation of immune responses against these pathogens, Langerin also mediates other processes that could be dependent on signaling. For example, Langerin protects from HIV-1 infection by internalization and degradation of HIV-1 [87]. This process might be mediated by LSP1 dependent signaling that targets HIV-1 to the proteasome, as described previously for DC-SIGN [10]. L-SIGN expression is predominantly found on lymph endothelium and liver sinusoidal endothelial cells (LSECs) [91], which are specialized endothelial cells localized between the liver sinusoids and hepatocytes. L-SIGN recognizes mycobacteria [92] and can mediate the transmission of several different viruses for productive infection of target cells, as described for HIV-1 [91], HCV [93] and Ebola [94]. Since very little is known about signaling of both Langerin- and L-SIGN-mediated processes, LSP1 seems to be the most suitable candidate for exploring signaling by these receptors.

Taken together, it seems clear that C-type lectins mediate several immunological functions that are most likely mediated through induction of signaling. Although for many C-type lectins the signaling pathways are still unknown, some pathways are shared by multiple receptors, which might ease our quest of unraveling the signaling pathways underlying C-type lectin-mediated functions.

Targeting of C-type lectin signaling for development of vaccine strategies

Besides offering great insights into the induction of immune responses against pathogens, the knowledge about the currently identified signaling pathways also provides promising tools for therapeutic purposes. Particularly, targeting of the C-type lectin signaling pathways could be of high potential interest for development and enhancement of vaccine strategies. Effective vaccines have been of tremendous importance for public health, as illustrated by the eradication of naturally occurring smallpox and the control of diseases such as polio, measles, rubella, mumps, chickenpox and typhoid. However, despite extensive efforts, for numerous pathogens including HIV, HCV and malaria still no vaccines are available, whereas for other pathogens such as *M. tuberculosis* vaccination only provides limited protection. As demonstrated in this thesis, C-type lectin signaling plays a crucial role in the induction of adaptive immune responses by DCs, which is essential for generating vaccine-induced

immunity. Hence, induction or inhibition of these pathways might be a powerful tool for generating specific immune responses that provide protective and long-lasting immunity.

The current vaccine against tuberculosis comprises inoculation with the live-attenuated mycobacterial strain *M. bovis* BCG. As mentioned before, efficacy of BCG vaccination is limited: although BCG effectively protects infants from tuberculosis, immunity declines with age and fails to protect adults against pulmonary tuberculosis, the primary source of dissemination [95-97]. Although almost all vaccines developed thus far against acute diseases function largely through the induction of specific antibodies [98], this might not be the ideal design for vaccines against intracellular pathogens such as *M. tuberculosis*, since the pathogen is protected from immune attack by antibodies within cells. Instead, protection requires mobilization of the cellular arm of the immune response mediated by T cells. Effective protection against *M. tuberculosis* requires T_H1, T_H17 and cytotoxic T cell responses [35-37]. Notably, both T_H1 and T_H17 [57] and cytotoxic T cell responses [99] can be induced through Syk and Raf-1 signaling. It would be interesting to evaluate whether the specific activation of these pathways could enhance vaccination efficacy, e.g. by implementation of Syk and Raf-1 activating ligands as adjuvants for enhancing the immunogenicity of vaccines against tuberculosis. In support of this theory, it has recently been shown that the induction of Syk signaling as an adjuvant for a tuberculosis subunit vaccine with a mycobacterial component generates T_H1 and T_H17 responses and partial protection against *M. tuberculosis*, albeit in a dectin-1-independent manner [100].

In addition, induction of C-type lectin signaling pathways might also be used for the specific induction of other T helper cell responses. Depending on the ligand, DC-SIGN induces three different signaling pathways resulting in three distinct cytokine profiles. Although the specific T helper responses resulting from these signaling pathways have not yet been thoroughly investigated, current studies suggest that the different signaling pathways induce distinct polarizations of the immune response: mannose-structures direct T cell polarization towards T_H1 (unpublished results), Salp15 skews towards T_H2 (MAWP de Jong, personal communications), and fucose-structures promote either T_H2 or T_{reg} responses [43], although the specific cytokine profile after fucose-stimulation seems to correlate with the latter (Chapter 4 and unpublished results). Since different ligands induce distinct T cell polarizations, the use of specific ligands as adjuvants might allow us to steer immune responses in the direction that generates optimal vaccine efficacy.

Targeting of C-type lectins with an antibody might also be a promising

tool for the induction of immunity. In the last years, several reports have described that targeting of C-type lectins on DCs with an antibody coupled to an antigen can lead to immune responses against a given antigen. Recently, two studies independently demonstrated that antigen targeting to CLEC9A (also known as DNGR-1) is a promising tool for vaccine development [80, 81]. Studies in mice showed that antigens coupled to CLEC9A antibodies strongly enhanced antibody production and CD4 and CD8 T cell responses, which could prevent the development and mediate eradication of melanoma metastases [81]. Since CLEC9A is also selectively expressed by a subset of human blood DCs [80, 81], targeting of this C-type lectin might also be used for the development of vaccine strategies in humans. Strikingly, targeting of CLEC9A induced adaptive immune responses even in absence of adjuvants [80], suggesting that signaling of this C-type lectin, which is mediated via Syk [77], is sufficient for immune activation. Since dectin-1 also signals via Syk, dectin-1 poses to be an interesting candidate for targeting. However, in order to study dectin-1 targeting activating antibodies should be developed first, because no such antibodies are available yet. Alternatively, cross-linking of dectin-1 with the currently available antibodies might also induce signaling, although definite proof for this is still lacking. In contrast to dectin-1, several activating antibodies are available for DC-SIGN [33, 101], but since DC-SIGN signaling alone does not induce NF-κB activation and subsequent DC maturation and cytokine induction, DC-SIGN antibodies would require an adjuvant for the induction of immune responses. A promising tool for the induction of signaling by targeting of DC-SIGN and dectin-1 could be the use of liposomes. Liposomes expressing antibodies or specific carbohydrates could deliver antigens to DCs resulting in processing and antigen presentation, while simultaneously triggering of the C-type lectins induces immune activation, which combined results in the induction of antigen-specific immune responses. In addition, varying the antibodies or carbohydrate structures on liposomes might allow us to control the activation of signaling pathways in DCs and thereby the subsequent polarization of T cell responses.

The possibilities of using C-type lectin signaling for polarization of immune responses are even extended when using DC vaccination, which has proven to be a promising technique for the treatment of cancer [102]. For DC vaccination, most clinical studies use monocytes-derived DCs that are loaded with antigen, are matured, and subsequently administered to the donor. Since in this approach DCs are cultured ex vivo, specific signaling pathways can not only be induced, but simultaneously specific signaling proteins could also be inhibited, for example by using chemical inhibitors or RNA interference. This would allow us to even further tailor

the immune response to the tumor or pathogen involved, resulting in highly effective adaptive immune responses.

Taken together, C-type lectin signaling pathways provide a promising target for new vaccination strategies. The most feasible approach might be the development of subunit vaccines with custom carbohydrate structures for the induction of specific C-type lectin signaling pathways and concordant T cell responses. In addition, targeting of C-type lectin signaling pathways, either by antibodies coupled to antigens or by liposomes, might also be an effective approach for the induction of immunity. DC vaccination provides even more possibilities, but since this is very laborious and expensive it will probably not be the first method of choice.

Concluding Remarks

The induction of immune responses against pathogens critically depends on innate signaling by PRRs such as C-type lectins. In this thesis the signaling pathways for two important C-type lectins, DC-SIGN and dectin-1, have been identified. These findings give new insights into how specific immune responses against pathogens are induced. DC-SIGN generates pathogen-specific immune responses via the induction of ligand-specific signaling pathways, which dependent on the microorganism can be beneficial for host or pathogen. In addition, dectin-1 induces specific T helper cell responses via the induction and crosstalk of two signaling cascades. The identification of these pathways might help unraveling signaling pathways of other C-type lectins or even other PRRs, since some of these signaling pathways are shared by multiple receptors. Eventually, these findings might lead to new therapeutical strategies for microbial infections, vaccination protocols or chronic inflammatory conditions.

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