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C-type lectin receptors in control of T helper cell differentiation

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Abstract

Pathogen recognition by C-type lectin receptors (CLRs) expressed by dendritic cells is not only important for antigen presentation, but also for induction of appropriate adaptive immune responses via T helper (T_H) cell differentiation. CLRs induce signaling pathways that trigger specialized T_H-specific cytokine programs either by themselves or in cooperation with other receptors such as Toll-like receptors and Interferon receptors. In this review, we discuss how triggering of the prototypic CLRs leads to distinct pathogen-tailored T_H responses and how we can harness our expanding knowledge for vaccine design and treatment of inflammatory and malignant diseases.

Efficient T cell responses require differentiation of $CD4^+$ T cells into different T helper (T_H) cell subsets, depending on the type of infection, which have specific roles in defense against pathogens, by helping other innate and adaptive immune cells in mounting effective defenses to the invading pathogens (FIG. 1). Antigen-presenting cells and in particular dendritc cells (DCs) orchestrate T_H cell differentiation by creating a specialized cytokine environment, in combination with TCR stimulation. The prime determinant controlling T_H cell differentiation is pathogen recognition by DCs, which translates the nature of the invading pathogen into a gene transcription program appropriate to the specific T_H cell response[1]. Several different T_H cell subsets have been identified that each have specific functions in adaptive immunity (BOX 1). T_H1 responses are important in host defense against intracellular pathogens via the secretion of IFNγ, but also IL-2, GM-CSF and TNF. T_H1 cells activate neutrophils to kill fungi, infected macrophages to kill intracellular *Mycobacterium tuberculosis* and $CD8^+$ T cells as well as natural killer cells to kill virus-infected cells[2]. IL-12p70, secreted by DCs and consisting of IL-12p35 and p40 subunits, is paramount to the induction of T_H1 cells via induction of transcription factor T-bet, which is considered to be the master regulator of T_H1 cell differentiation[3]. T_H2 responses are required as defense against extracellular pathogens by activating eosinophils and basophils, and by inducing antibody isotype switching to IgE in B cells, via secretion of IL-4, IL-5 and IL-13[4]. DCs are important inducers of T_H2 responses, but the mechanisms are not completely understood. The differentiation of T_H2 cells is reciprocally entwined with T_H1 differentiation, as inhibition of IL-12 secretion by DCs facilitates T_H2 differentiation, while the induction of costimulatory molecule OX40L also primes DCs towards T_H2 polarization[5,6]. Follicular T helper (T_{FH}) responses are essential for the establishment of protective long-term humoral immunity against pathogens via the generation of high-affinity antibodies, via secretion of IL-21[7]. Multiple signals act in concert for the initiation of T_{FH} differentiation at the time of DC priming, with IL-6 and IL-21 having important roles *in vivo* in the generation and maintenance of T_{FH} cells and formation of germinal centers in mice[7]. Recently it was shown that IL-27 is also crucial for inducing human T_{FH} differentiation[8], while T_{FH} generation is impaired in IL-27R knock-out mice[9]. T_H-17 responses are crucial in the defense against fungi, via secretion of the hallmark cytokines IL-17A, IL-17F and IL-22 that recruit neutrophils to sites of infection[10]. Expression of transcription factor RORγT, the master regulator of T_H-17 cells[11] is induced in activated human $CD4^+$ T cells via simultaneous IL-6 and IL-1β stimulation, while IL-23, consisting of subunits IL-12p40 and IL-23p19, plays an important role in the maintenance of T_H-17 commitment[12]. Mice T_H-17 polarization is more dependent on TGFβ besides IL-6 and IL-1β[11]. Several other T_H cell subsets help in defense against pathogens, e.g. T_H9 and T_H22[13,14], while also a lot of plasticity exists

between different T_H subsets. For example, T_H22 cells are distinct from T_H-17 and T_H1 subsets due to their production of IL-22, but not IL-17 or IFNγ[15] Furthermore, regulatory T cells are important to limit immune responses and thereby prevent autoimmune and other deleterious immune reactions[16].

In this review, we focus on the role of C-type lectin receptors (CLRs) that function as pattern recognition receptors (PRRs) on the cell surface in the induction of T_H responses. We integrate our current understanding of CLR signaling pathways that control the specialized cytokine expression profiles upon sensing of invading pathogens with the role of specific T_H responses in infection, and how we can harness this to conceive more straightforward vaccination strategies to combat not only infectious diseases, but also autoimmune diseases and malignant disorders.

C-type lectin receptors and T_H cell differentiation

CLRs comprise of a large superfamily of proteins that are defined by the presence of at least one C-type lectin recognition domain[17]. The entire CLR family recognizes a diverse range of carbohydrate structures such as mannose, fucose, sialic acid and β-glucan, which dictates the pathogen specificity of the CLRs[18]. CLRs are expressed by myeloid cells and in particular by macrophages and DC subsets, but some CLRs, such as dectin-1, are also expressed by neutrophils or B cells or even keratinocytes[19,20]. The expression of CLRs on DC subsets dictates how these DC subsets react to pathogens and also underlies their specialized functions. Diversification and division of labour between these DC subsets is important in immune defense[21]. Therefore, it is not very surprising that some CLRs have a very restrictive expression pattern, such as langerin or CLEC9A[22,23]. CLRs differ from most other PRRs in that pathogen recognition via high affinity and avidity interactions leads to uptake of pathogens and eventually degradation in the antigen processing machinery, while it also leads to induction of innate signaling that induces or modulates innate and adaptive immunity[18]. Many CLRs are effective PRRs that are able to sense different classes of pathogens as well as malignant tumor cells by their glycosylation pattern, or so called carbohydrate fingerprint[18]. Bacteria, parasites and fungi have glycosylation structures that are distinct from host carbohydrates. Interestingly, even though viruses hijack the glycosylation machinery of host cells, their glycosylated envelope proteins differ from endogenous proteins in amount of glycosylation as well as composition, i.e. high mannose structures are more prevalent than complex and hybrid glycans[24]. Malignant cell formation also causes distinct activation and/or expression of glycosyltransferases, resulting often in tumor-specific glycosylation patterns, such as Tn antigens[25]. This makes CLRs

exceptionally well equipped to recognize and distinguish between different pathogens and tumor cells and orchestrate the required appropriate adaptive immune responses.

Dectin-1 induces antifungal T_H1 and T_H-17 responses

A prototypic CLR is dectin-1 that is crucial in defense against fungi, as underscored by the increased susceptibility to *Candida* infections of persons with a defective dectin-1 protein[26]. Similarly, dectin-1-deficient mice are less resistant to various fungal infections[27,28]. As shown in mice, dectin-1 is also important in maintaining microbiota homeostasis in the colon by controlling growth of the resident fungi[29]. Dectin-1 recognizes β-glucans exposed at budding scars of pathogenic as well as opportunistic fungi (TABLE 1), and thereby holds the key in unlocking antifungal immune responses. Dectin-1 is expressed on epithelial LCs as well as subepithelial DCs, which positions it ideally for the detection of invasive fungi. While LCs are important in the induction of T_H-17 responses to cuteanous fungal infections[18,30], DCs induce both T_H-17 and T_H1 responses[30-32], where the latter is important in defense against systemic invasive fungal infection[30], exemplifying the division of labour between LCs and DCs. Interestingly, dectin-1 signalling is only activated by particulate β-glucans, which cluster the receptor in synapse-like structures[33]. Early studies showed that dectin-1 collaborates with signaling by TLR2 to enhance cytokine responses in macrophages[34]. However, dectin-1 is in its own right crucial in the induction of adaptive immunity by triggering several distinct signaling pathways that cooperate to shape T_H responses in response to fungal infestations[31,32,35,36].

Dectin-1 ligand binding and subsequent receptor clustering (BOX 1) triggers phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM)-like sequence within their cytoplasmic domains, leading to consecutive recruitment of SHP2 and tyrosine kinase Syk and formation of a CARD9-Bcl-10-Malt1 scaffold[31,32,35-37]. This scaffold initiates a signaling cascade that leads to activation of both the classical and non-canonical NF-κB pathways, i.e. activation of classical p65/p50, c-Rel/p50 and non-canonical RelB/p52 transcriptional complexes[31]. p65/p50 and c-rel/p50 complexes trigger expression of IL-6 and IL-23p19, necessary for T_H-17 immune responses, by binding to elements within the transcriptional promoter/enhancer regions of their respective genes, *IL6* and *IL23A*[31]. Expression of IL-1β and IL-12p40 is equally essential to T_H-17 induction, but requires activation of a second Syk-independent pathway by dectin-1 that is mediated by the serine/threonine kinase Raf-1. Raf-1 activation overcomes the repressive effects of RelB/p52 complexes on *IL1B and IL12B* transcription by inducing phosphorylation and acetylation of Syk-induced p65 at Ser276[38] which leads to formation of an inactive RelB-p65 dimer, thereby attenuating the repressive effects of RelB/p52 and allowing p65/p50-induced

transcriptional activation of *IL1B* and *IL12B*[31]. Cooperation between the distinct Syk- and Raf-1-dependent signaling pathways induced via fungal recognition by dectin-1 results in a cytokine environment that directs T_H-17 differentiation[31].

Since aberrant T_H-17 responses are associated with pathogenic hyperinflammatory disorders[12], it is important to control T_H-17 differentiation closely. Given its role in T_H-17 polarization, the expression of IL-1β is tightly regulated. First, transcription of *IL1B* is carefully controlled and balanced, not just through controling activation of different NF-κB complexes, as described above, but also via autocrine IFN receptor signaling (BOX 1). Dectin-1 recognition by *C. albicans* triggers IFNβ expression via Syk-dependent IRF5 activation[39], resulting in a type I IFN expression profile[40], but also in downregulation of IL-1β expression and T_H-17 responses[41]. A second checkpoint is the secretion of IL-1β, since immature pro-IL-1β protein requires enzymatic processing to generate biologically active IL-1β for secretion[42]. Many pathogens, including fungi, activate an additional cytosolic receptor like NLRP3 to trigger activation of caspase-1 inflammasomes that cleave pro-IL-1β[43]. Recent studies show that dectin-1 signaling itself also controls the processing of pro-IL-1β, through activation of the non-canonical caspase-8 inflammasome[44-47] (BOX 1). Activation of the caspase-8 inflammasome is directly integrated in the dectin-1 signaling pathway and independent of pathogen internalization[44]. Some fungi also trigger canonical caspase-1 inflammasomes via dectin-1, which is dependent on fungal internalization as well as radical oxygen species production and potassium efflux[44,44,48,49]. A further level of control over T_H-17 differentiation is manifested via expression of the interferon-stimulated gene (ISG) IL-27 that occurs as a result of dectin-1-induced type I IFN[41], thereby blocking T_H-17 development[50].

Cooperative Syk and Raf-1 signaling after dectin-1 triggering also leads to antifungal T_H1 responses that are dependent on expression of IL-12p70[31] (BOX 1). Transcription of *IL12A*, encoding subunit IL-12p35, is crucially dependent on chromatin remodeling to allow binding of transcriptional activators such as NF-κB[51]. Fungal recognition by dectin-1 activates transcription factor IRF1 via Syk-dependent signaling, which is essential for nucleosome remodeling at the *IL12A* promoter[51]. Inhibition of IRF1 activation leads to the loss of IL-12p70 expression and skews T_H cell differentiation towards T_H2 responses[51], which are detrimental for antifungal immunity[52]. Next, transcription of both IL-12 subunits, p35 and p40, is mediated by c-Rel/p50 and p65/p50 complexes and again prolonged due to Raf-1-induced phosphorylation and acetylation of p65, as described above[31]. Inhibition of Raf-1 signaling shifts T_H1 responses in response to dectin-1 triggering toward T_H2 responses[31]. Also in neonatal DCs, dectin-1 sigaling via Syk and Raf-1 induces T_H1 responses by unlocking *IL12A* transcriptional control[53]. Murine

dectin-1 signaling also leads to reduced expression of OX40L, thereby shifting T_H responses away from T_H2 differentiation[54] Raf-1 activation by dectin-1 has furthermore been shown to induce epigenetic reprogramming of monocytes, thereby enhancing innate antifungal immunity by monocytes upon second exposure[55], and it is conceivable that this process is also important in reinforcing adaptive immunity. Collectively, these observations show that intricate cooperation between signaling pathways induced by dectin-1 itself is essential for shaping antifungal T_H responses and underscore the potential of targeting these signaling pathways in vaccinations.

Carbohydrate-specific T_H responses by DC-SIGN

DC-SIGN expressed on subepithelial DCs and macrophages has been most extensively studied and is the most versatile of CLRs[18] (TABLE 1). Although DC-SIGN is unable to induce cytokine expression on its own, its signaling pathways modulate signaling induced by other PRRs in a carbohydrate-specific manner, thereby differentiating T_H responses between pathogens. While many intracellular pathogens, such as mycobacteria, viruses as well as fungi bind to DC-SIGN via mannose-containing structures, DC-SIGN equally recognizes extracellular pathogens, like parasites and *Helicobacter pylori*, via fucose-carrying pathogen-associated molecular patterns (PAMPs)[18]. All of these pathogens trigger various PRRs simultaneously, including TLRs and/or cytosolic receptors, besides DC-SIGN. It is the cooperation between signaling by these PRRs and DC-SIGN that promotes either T_H1/T_H-17 or T_H2/T_{FH} responses to combat either intracellular or extracellular pathogens, respectively.

DC-SIGN-mannose interactions direct T_H1 and T_H-17 responses.

DC-SIGN recognizes a diverse array of mannose PAMPs; *Mycobacterium tuberculosis* as well as *M. leprae* express lipoarabinomannan structures containing a mannose-cap (ManLAM), whereas many different viruses including HIV-1, Ebola and HCV have envelope glycoproteins that contain high mannose structures, while the cell wall of many different fungi contains the polycarbohydrate mannan[18] (TABLE 1). These pathogens trigger PRRs such as TLR2, TLR4 or dectin-1, thereby activating p65/p50 transcriptional complexes and subsequently a cytokine expression program resulting in transcription of pro-inflammatory cytokines such as IL-12p35, IL-12p40 and IL-6 is initiated[31,38,56]. Simultaneous recognition of these pathogens by DC-SIGN results in the activation of Raf-1[38,56,57], as occurs for dectin-1, and subsequent phosphorylation and acetylation of NF-κB subunit p65 at Ser276[38], as discussed above, enhances TLR-induced transcription of *IL12A, IL12B* and *IL6* (BOX 2). The increased IL-12p70 secretion by DCs skews T_H cell differentiation towards T_H1 responses[31]. In mice, binding of DC-SIGN homologue

SIGNR3 similarly activates Raf-1 during infection with *M. tuberculosis*, and SIGNR3 signaling cooperates with TLR2 signaling to induce pro-inflammatory cytokine expression and protective immunity[58]. During fungal infections, both dectin-1 and DC-SIGN signaling is triggered, and the additional Raf-1 activation by DC-SIGN strengthens not only T_H1 but also T_H-17 responses through upregulation of IL-6, IL-1β and IL-23[31,56] (BOX 2).

DC-SIGN-fucose interactions direct T_H2 and T_{FH} responses

Fucose-containing ligands program a completely different cytokine expression profile via DC-SIGN than mannose PAMPs to elicit adaptive T_H2 and T_{FH} responses, resulting in humoral immunity[8,59]. These differences in cytokine programs likely reflect differences in mannose and fuocse binding to the carbohydrate recognition domain of DC-SIGN[60]. Fucose binding induces conformational changes that lead to displacement of the so-called Raf-1 signalosome from DC-SIGN, enabling the assembly of a different signaling complex[59] (BOX 2). DC-SIGN recognizes a wide range of fucose PAMPs, such as LewisX antigen (LeX) on helminth *Schistosoma mansoni*, LDNF on flatworm *Fasciola hepatica*, but also LeY on gastric pathogen *H. pylori*[8,18]. *H. pylori* spontaneously expresses LPS with or without LeY at different phases, where fucose-containing LPS induced T_H2 responses in contrast to fucose-negative LPS[61] (TABLE 1).

Similar to mannose signaling by DC-SIGN, fucose PAMPs are unable to induce cytokine expression by themselves. Invading microbes with fucose PAMPs trigger other PRRs, such as TLR2, TLR3 or RIG-I-like receptors (RLRs), and DC-SIGN-dependent immune responses are dependent on cooperation with the simultaneous signaling by these PRRs[8,59]. Displacement of the Raf-1 signalosome from DC-SIGN after recognition of fucose-containing ligands is followed by assembly of a fucose-specific signalosome, consisting of serine/threonine kinase IKKε and de-ubiquitinase CYLD, which leads to the activation and nuclear translocation of an atypical NF-κB family member, Bcl3[59] (BOX 2). Bcl3 by itself is unable to promote or repress transcription, however it forms complexes with p50/p50 dimers that have a high affinity for DNA, thereby replacing TLR- or RLR-induced p65/p50 transcriptional complexes from promoter regions[62]. The effects of Bcl3/p50/p50 complexes on transcription depends on the context of the entire promoter[63,64]; binding of Bcl3/p50/p50 complexes to either the *IL12A* or *IL12B* promoter represses their transcriptional activation, and as such represses IL-12p70 expression, which results in skewing of T_H responses away from T_H1 and towards a T_H2 profile[59]. T_H2 cell differentiation is further promoted by the expression of two T_H2-attracting chemokines, CCL17 and CCL22, which is positively regulated by Bcl3/p50/p50 complexes[59] (BOX 2). Binding of specific fucose PAMPs from different parasites, i.e. *S. mansoni* and *F. hepatica,* as well as *H.*

pylori induces T_H2 responses in a Bcl3-dependent manner[8]. *S mansoni* PAMPs also induce OX40L expression, a marker for DCs primed for T_H2 differentiation, possibly via DC-SIGN[65].

Besides programming DCs to create a cytokine environment that promotes T_H2 differentiation, fucose-specific DC-SIGN signaling also leads to the expression of cytokines that directs $CD4^+$ T cells towards T_{FH} differentiation, i.e. IL-6 and IL-27[8] (BOX 2). Notably, the expression of these cytokines is independent of Bcl3 activation, but depends on crosstalk with IFN receptor signaling, which is triggered upon IFNβ release as a result of pathogen interactions with other PRRs[8]. IKKε, as part of the assembled fucose-specific signalosome, cooperates with IFN receptor signaling to induce formation of the ISGF3 transcriptional complex[8]. ISGF3 positively regulates the expression of IL-27 subunit IL-27p28, thereby crossing the threshold for the amount of IL-27 required for directing T_{FH} differentiation[8]. Interfering with ISGF3 activation after triggering fucose-specific DC-SIGN signaling completely blocked T_{FH} differentiation[8].

Simultaneous induction of both T_H2 and T_{FH} responses by parasitic fucose PAMPs via DC-SIGN, with a central role for IKKε in both responses, leads to robust protective long-term humoral immunity. Both DC-SIGN-induced T_H subsets play distinct roles in the generation of protective long-term humoral immunity against parasites. T_{FH}-produced IL-21 acts as a switch factor for human IgG1 and IgG3 within GCs, thus inducing $IgG1^+$ B memory cells[66]. Eosinophil-mediated killing of parasites depends on the coating of the parasite with antigen-specific IgE antibodies, while high-affinity IgE production is also essential for the expulsion of intestinal parasites[67]. The development of high-affinity IgE antibodies relies on a sequential class switching from intermediate $IgG1^+$ memory B cells to long-lived high-affinity IgE^+ plasma cells[68], outside GCs[69], which is strictly dependent on T_H2-produced IL-4[70]. Even *in vitro* differentiated T_H cells upon priming of human DCs with fucose PAMPs provide cognate help to B cells and induce class switching, production of IgG and prolonged survival[8]. Thus, although the crosstalk between fucose-specific DC-SIGN and other PRRs leading to complementary T_H responses is complex, understanding at the molecular level makes manipulation of these pathways a powerful target in vaccination strategies, not just towards parasitic infections, but also towards infections such as HIV-1, where broadly neutralizing antibodies require T_{FH} responses[71]. Additionally, fucose-specific signaling by $DC\text{-}SIGN^+$ macrophages, like by DCs[56,59], strongly upregulates IL-10 expression, which results in induction of regulatory T cells[72].

Dectin-2 promotes antifungal T_H-17 and allergic T_H2 responses

Unlike dectin-1 or DC-SIGN, CLR dectin-2 is unable to signal by itself, instead associating with the signaling adapter FcRγ chain that induces signaling via its ITAM[73]. In mice, dectin-2-FcRγ

complexes are also thought to form functional complexes with the related CLR MCL[74]. Dectin-2 is expressed on a variety of myeloid cells, including macrophages and several DC subsets where it interacts with α-1,2-mannose structures on fungi[75] as well as mycobacterial ManLAM[76] (TABLE 1). Recognition of fungal pathogens by human dectin-2 triggers signaling via FcRγ; like dectin-1, Syk-dependent CARD9-Bcl-10-Malt1 scaffolding formation is induced, however, unlike dectin-1, only c-Rel/p50 transcriptional complexes are activated[77] (BOX 3). As such, human dectin-2 is unable to induce cytokine expression on its own, but it further supports expression of c-Rel-controlled genes, such as IL-23p19 and IL-1β, thereby strongly contributing to T_H-17 responses, in cooperation with dectin-1 signaling[77-80]. It is undetermined whether dectin-2 is complexed with MCL in these circumstances. Human dectin-2 also supports the induction of IL-1β in DCs and TNF in both monocytes and DCs by *M. bovis* BCG, although it was not investigated whether dectin-2 could induce these cytokines on its own and how this affects T_H differentiation[76]. In contrast, triggering of murine dectin-2 by α-mannoses from *C. albicans* hyphae leads to Syk- and CARD9-Bcl-10-Malt1-dependent NF-κB activation[78,81] – p65 complexes specifically, although other subunits were not directly studied[78] – and subsequent expression of cytokines, such as TNF, IL-10, IL-6, IL-23, IL-12, IL-1β[78,80,81] and T_H-17 responses[78,80] (BOX 3). Murine dectin-2 signaling also promotes dectin-1-dependent T_H1 responses in response to fungal PAMPs, but is unable to instigate those by itself, and it remains to be determined which cytokines are involved in this effect[78,80]. Furthermore, murine dectin-2 induces expression of proinflammatory cytokines such as TNF, IL-6 and IL-23, as well as anti-inflammatory cytokines IL-10 and IL-2 in response to mycobacterial ManLAM, thereby promoting T_H-17 responses[76]. Interestingly, recognition of *S. mansoni* PAMPs by murine dectin-2 fails to induce *IL1B* transcription, however, it does result in Syk-dependent radical oxygen species production and potassium efflux to activate the NLRP3-caspase-1 inflammasome to process immature pro-IL-1β produced in response to triggering of other PRRs, thereby influencing T_H-17 responses[82]. It remains unclear whether the reported differences between murine and human dectin-2 signaling reflect species differences or are due to cellular background as different DC subsets were studied, e.g. bone marrow-derived vs monocyte-derived DCs.

Murine dectin-2 has also been shown to interact with glycans on allergens from *Aspergillus fumigatus* and house dust mites *Dermatophagoides farinae* (Df) and *D. pteronyssinus*, thereby inducing signaling that skews T_H cell differentiation towards T_H2 responses[83-85] (TABLE 1). Recognition of Df allergens by dectin-2 leads to Syk-dependent cysteine-leukotriene production[84,85], which can counteract IL-12 expression[86], thereby inducing T_H2 responses[84] (BOX 3). Notably, sensitization of dectin-2-deficient mice with Df allergens did not induce eosinophilic

and neutrophilic pulmonary inflammation and T_H2 responses in lymph nodes and lungs[83]. Thus, dectin-2 control of T helper cell differentiation varies with species and ligands, which might be harnessed to direct immunological responses during vaccination strategies.

The role of mincle in T_H1 and T_H-17 responses

CLR mincle was first identified as a sensor of cell death[87], but soon its potential in modulating adaptive immune responses was uncovered. Mincle is expessed on DCs and macrophages, but also neutrophils and some B cell subsets, where it recognizes carbohydrates on various pathogens including pathogenic *Fonsecaea* fungi as well as *M. tuberculosis*[51,79,88,89]. Mincle binds to mycobacterial cord factor, a glycolipid, via its trehalose structure[90], while it recognizes glycolipids from *Malassezia* species via α-mannosyl and glucosyl structures[75] (TABLE 1). Like dectin-2, mincle requires association with the FcRγ chain for signaling[87]. There is a remarkable difference in the role of mincle in adaptive immunity between human and mice, almost exhibiting opposing behaviour, with murine mincle promoting antifungal T_H1 responses[89,91], whereas human mincle opposes T_H1 immunity[51], and variable T_H-17 responses[79,89,92,93]. These differences might reflect differences between expression and cooperation of human and murine mincle with MCL, another CLR. Expression of mincle on immature murine DCs is low, but is induced by various stimuli, including MCL triggering, and subsequently leads to formation of MCL-mincle-FcRγ complexes that are required for the induction of cytokine expression and immune responses[92,94,95,95]. In contrast, immature human DCs exhibit abundant expression of mincle as well as MCL[51,95]. Also, signaling via human mincle in response to *Fonsecea* species or mycobacterial cord factor is independent on MCL[51]. This is further confirmed by Ostrop *et al.,* who show that retroviral expression of human MCL does not confer mycobacterial cord factor responsiveness to murine mincle-deficient bone marrow-derived DCs, while co-expression of human MCL with human mincle does not further enhance human mincle-induced responses[95]. Interestingly, a recent study identifies cholesterol crystals as a ligand for human mincle, but neither for murine mincle nor human or murine MCL[96].

In murine models, recognition of mycobacterial cord factor by mincle together with MCL induces signaling via the FcRγ chain that, like dectin-1, results in Syk-dependent CARD9-Bcl-10-Malt1 scaffold formation, subsequent NF-κB activation and cytokine expression that leads to protective T_H1 immunity[89,93] (BOX 4). As such, mincle contributes to the control of splenic *M. bovis* BCG infection in mice[97]. In contrast, triggering of human mincle on DCs by either pathogenic *Fonseceae* fungi, the causative agents of the chronic skin infection chromoblastomycosis[98], or mycobacterial cord factor leads to evasion of protective T_H1 immunity by evoking T_H2 immunity[51]

(BOX 4). Notably, human mincle-FcRγ signaling on DCs by *Fonseceae* species or mycobacterial cord factor results in Syk-dependent CARD9-Bcl-10-Malt1 scaffold formation, but not NF-κB activation and cytokine gene expression[51] (BOX 4). Although mycobacterial cord factor recognition by human macrophages leads to Syk- and CARD9-dependent IL-8 production, mincle is not required for this response[95]. Instead, mincle triggering leads to Syk-dependent activation of the E3-ubiquitin ligase Mdm2[51]. As described above, *IL12A* transcriptional activation requires chromatin remodeling via IRF1 binding, which is induced by *Fonseceae* species through dectin-1[51]. However, activation of Mdm2 by mincle triggering effectively blocks *IL12A* transcription, as Mdm2 targets nuclear IRF1 for proteasomal degradation, thereby suppressing IL-12p70 expression and skewing T_H cell differentiation towards T_H2 responses[51]. T_H2-biased immunity causes severe suppression of phagocytic effector cell function and hence has adverse effects on fungal infections[52], which manifests in chromoblastomycosis patients as a high fungal load in lesions that are characterized by the presence of T_H2 cells[99]. Also, in a murine chromoblastomycosis model, co-stimulation of TLR enabled mincle-dependent protective immunity against *Fonseceae pedrosoi*[100], while triggering of human mincle represses TLR4 signaling, similar to dectin-1 signaling[51] (BOX 4). Recognition of cholesterol crystals by human DCs induces expression of proinflammtory cytokines, which is partially dependent on mincle triggering, suggesting that human mincle signaling can not only interfere with transcription, but also enhance it through cross-talk with signaling of other PRRs[96]. It will be interesting to determine whether this positive cross-talk depends on the nature of the other PRR(s) – cholesterol crystals are not recognized by either dectin-1 or dectin-2[96] – or on the targeted genes. It is undetermined how T_H responses are affected by human mincle recognition of cholesterol crystals.

The induction of T_H-17 immunity seems to vary not just between human and mice, but also with the nature of the mincle ligand. Activation of murine mincle by mycobacterial cord factor leads to expression of IL-6, IL-23 and pro-IL-1β[89,92,93] (BOX 4), whereas processing of pro-IL-1β is induced via mincle/Syk-dependent activation of the caspase-1 inflammasome via NLRP3[101], collectively resulting in T_H-17 immunity. Surprisingly, mincle triggering in mice during *F. pedrosoi* infection suppresses dectin-2-mediated T_H-17 cell differentiation, although the mechanism behind this response remains elusive[79]. In contrast, human mincle signaling promotes T_H-17 responses by attenuating type I IFN responses. Mincle-induced activation of Mdm2 does not just lead to degradation of IRF1, but also of IRF5, the transcriptional activator of *IFNB*[41]. As described above, IFNβ affects *IL1B* transcription and IL-27 expression, hence reduced IFNβ levels increase expression of T_H-17-polarizing IL-1β and lower expression of T_H-17-dampening

IL-27, with a net effect of increased T_H-17 immunity[41]. Collectively, these observations show the opportunities of harnessing mincle-mediated adaptive immune responses for successful vaccinations, as has been shown in mice[93,97], but more importantly, exemplify the differences in human and mice immunity, instigating caution when extrapolating results obtained in murine models to human diseases and vaccination strategies.

Diversification with common signaling scaffolds

While many CLRs that function as PRRs signal via Syk-mediated CARD9-Bcl-10-Malt1 scaffolds, triggering by pathogens often has very distinct immunological outcomes; while dectin-1 signaling initiates T_H1 and T_H-17 responses, human dectin-2 only supports T_H-17 differentiation, while human dectin-2 and mincle seem to suppress T_H1 responses, as described above. An intriguing question is how these receptors induce distinct signaling pathways through a common signaling scaffold. Of course, dectin-1 differs vastly from FcRγ-mediated dectin-2 and mincle signaling as it also triggers LSP1-Raf-1-dependent signaling. While research into this route has mainly focused on Raf-1 activation and its effect on NF-κB activation, LSP1 either dependently or independently of Raf-1 could have various effects on other signaling pathways downstream from Syk and CARD9-Bcl-10-Malt1, which would set it apart from dectin-2/FcRγ and mincle/FcRγ signaling. For example, human dectin-1 is coupled to TRAF2 and TRAF6 recruitment to the CARD9-Bcl-10-Malt1 scaffold, while human dectin-2 only recruits TRAF2 (SI Gringhuis, TBH Geijtenbeek, unpublished observations), which might reflect a difference in cross-talk with LSP1-mediated signaling, and explain why dectin-1 signaling leads to activation of both classical p65/p50 and c-Rel/p50 NF-κB complexes, while dectin-2 only activates c-Rel/p50 complexes. Differences in complex formation of CLRs might also influence downstream signaling. While dectin-1 is thought to form dimers, dectin-2 and mincle are coupled to the FcRγ chain, but also capable of forming functional complexes with other receptors such as MCL. This might affect their abilities to form proximal signaling complexes. As FcRγ chains preferentially form dimers, the lack of association between human mincle and MCL might underlie the inability of human mincle to induce NF-κB activation; while human mincle might still induce formation of a CARD9-Bcl-10-Malt1 scaffold, its conformation might be inefficient in activating the necessary TRAF/TAK complexes for classical NF-κB activation, while at the same time allowing PI3K activation. Vice versa, dimerization of dectin-2 with MCL might allow FcRγ dimerization and subsequently efficient CARD9-Bcl-10-Malt1 scaffold formation and downstream NF-κB activaton. Deeper insights into the molecular build-up of the CARD9-Bcl-10-Malt1 scaffold and downstream signaling complexes will hopefully provide answers to these intriguing questions.

Other CLRs in T_H cell differentiation

Several other CLRs have also been shown to induce or inhibit adaptive immunity, but the underlying mechanisms are unknown. For example, the human mannose receptor (MR) contributes to antifungal T_H-17 immunity[102], while recognition of different allergens by MR results in downregulation of TLR-induced IL-12p70 expression, thereby favoring T_H2 responses[103]. MGL engagement enhances TLR-induced IL-10 expression, resulting in the induction of regulatory T cells[104,105]. CLEC9A in mice, is involved in the induction of antigen-specific T_{FH} cells[106], however, this receptor seems more important for cross-presentation than production of pro-inflammatory cytokines[107,108], suggesting that cooperation between CLEC9A and other receptors might underlie the observed effects on T_{FH} responses. Over the next few years, it will become clear whether CLR engagement is also involved in the induction of other specialized T_H subsets, such as T_H9 and T_H22.

Harnessing C-type lectin receptors for vaccination design

Live attenuated viruses, inactivated viruses, subunit vaccines that contain antigenic parts of a pathogen, or protein-polysaccharide-conjugated vaccines are currently the most used vaccines to protective mmunity[109]. However, there is an urgent need for vaccines that protect against infections such as HIV-1 and malaria, or emerging threats such as Ebola, but also against malignant disorders. The development of such vaccines has met with many difficulties and complications due to the complexity of the diseases, e.g. HIV-1 variability and its ability to escape immune responses, tolerance to cancer, or immune deficiencies in HIV-1-infected individuals[110]. Although most successful vaccines confer protection through neutralizing antibodies, it is becoming clear that cell-mediated immunity is equally important; for example T cell responses correlate better than antibody responses with protection by vaccines against varicella and influenza viruses[111,112]. It might be that both T and B cell responses are necessary for vaccines against specific pathogens such as HIV-1. Our increased understanding of the mechanisms by which CLRs create specialized environments to mediate distinct T_H cell subsets that coordinate both CD8 T cell and B cell responses allows for a novel level of sophistication in the design of vaccines.

CLRs have long been studied as targets for vaccination[113]. Targeting of CLR DEC-205 via an antibody linked to an antigen induces antigen-specific CD4 T cells and CD8 T cells[114-116]. The presence or absence of adjuvants, such as TLR ligands or CD40L, determines whether these antigen responses induce either immunity or tolerance, respectively[114,115]. In these and other

studies, the focus is mainly on the antigen presentation ability of CLRs and not on the immune function of CLRs, even though CLRs are very powerful PRRs, controlling immunity to many different pathogens.

In this review, we have discussed the different signaling pathways triggered by ligand recognition by CLRs, leading to specific T_H cell differentiation. There is a large diversity in the possible immune responses induced by CLRs that depends on different factors, such as type of CLR, nature of the carbohydrate ligand, and importantly, also the identity of the receptor(s) that are simultaneously triggered, like another PRR or even other receptors such as IFN receptors. It is this diversity that suggests that it should be possible to target CLR signaling to enhance/promote any type of T_H response and also CD8 T cell and antibody responses for vaccines (FIG. 2).

Antibodies are very useful in targeting CLRs for enhancing antigen presentation, but their ability to trigger CLR signaling has only been marginally investigated. A few antibodies are known to induce CLR signaling in soluble form, e.g. CLEC9A antibody 24/04-10B4 has been shown to induce T_{FH} responses[106], whereas a polyclonal antibody against DC-SIGN, H-200, triggers mannose signaling by DC-SIGN[117]. The high specificity of antibodies underscores their possible application in both targeting and triggering of CLRs.

As discussed and shown in TABLE 1, recognition of pathogens by CLRs leads to specific immune responses. However, since pathogens will trigger different CLRs and also other PRRs simultaneously, and the crosstalk between receptor signaling being paramount to CLR-induced immunity, the elicited immune responses are difficult to interpret, also depending on the DC subsets involved, as PRR expression will differ. For example, whereas one fungal species might trigger dectin-1 and dectin-2, thereby inducing protective T_H1 and T_H-17 responses, other fungi might trigger dectin-1 and mincle, leading to profungal T_H2 and limited T_H-17 responses[51,77]. Although whole pathogens have been used successfully in design of vaccins, such as *M. bovis* BCG and live attenuated viruses[109], the variety of CLR/PRR crosstalk predicts that this strategy will not work for more complex pathogens, or tumors.

A way around the use of whole pathogens might be the use of single carbohydrate structures as CLR ligands to elicit specific T_H responses to protect against infections or ameliorate inflammatory diseases. β-glucans are good candidates as they are specifically recognized by dectin-1 and induce protective T_H1 and T_H-17 immunity to *C. albicans* infections[27,31,32,35]. β-glucans have been successfully used to enhance antitumor responses, confirming their potential[118]. A drawback of using single carbohydrate structures is the fact that many CLRs have overlapping binding specificities[18], which does not allow for targeting of one specific CLR and

potentially inducing distinct, even contrasting immune responses when several CLRs are triggered.

Finally, microbial glycoproteins or glycolipids might be used to target and trigger CLRs on DCs. Mycobacterial cord factor is a glycolipid that seems specific for mincle/MCL and has successfully been used in mice for vaccinations against mycobacterial infections[93,97]. However, as discussed above, differences between murine and human mincle suggest that mycobacterial cord factor might act differently in human[51]. Also, viral envelope glycoproteins have distinct carbohydrate structures that interact with different CLRs such as DC-SIGN, langerin, DCIR and MR and thereby not only mediate infection of the cells, but also modulate innate signaling shaping immunity when used in combination with TLR ligands[38,56,119]. Helminth parasites express a large variety of glycoproteins, glycolipids as well as carbohydrates that can trigger specific immune responses. These effects could explain the beneficial use of helminth eggs in anti-inflammatory treatment of Crohns disease[120].

Many CLRs prompt efficient T cell immunity to a variety of carbohydrate structures, with DC-SIGN standing out due to its fucose-specific signaling, which promotes both T_H lineages that are required to elicit efficient B cell-mediated immunity[8]. Recent studies have shown that broadly neutralizing antibodies against HIV-1 can be protective against HIV-1 infection[121], however they show high mutation levels, indicating that many rounds of selection in germinal centers are necessary to form these broadly neutralizing antibodies[71]. Theoretically, a vaccine against HIV-1 that simultaneously triggers both fucose-specific DC-SIGN signaling and TLR-mediated IFN signaling should induce broadly neutralizing antibodies against HIV-1 by strong T_{FH} responses. Similarly, targeting of CLEC9A might also contribute to strong T_{FH} responses[106]. Thus, targeting of CLRs might also pose an important opportunity in the induction of strong antibody responses to HIV-1 infection.

Tumor research has focused on targeting CLRs for enhancing antigen presentation and in particular cross-presentation, which is paramount to activate CD8 T cell responses[110,122], but has hardly considered targeting CLRs for their immunomodulatory traits to enhance the induction of antigen-specific CD8 T cells. Interestingly, tumors exhibit aberrant glycosylation, which seems to modulate immune responses, i.e. certain tumors express fucose-containing antigens or sialic acids that suppress immunity[123,124]. Thus, a vaccine aimed to counteract the immunosuppressive tumor environment might offer an effective strategy. For example, induction of T_H1 responses in combination with cross-presentation might be achieved by the use of antibodies against CLRs that induce T_H1 responses or mannose-containing carbohydrates in specific liposomes carrying TLR ligands/adjuvants. Indeed, targeting of dectin-1 that enhances T_H1 and T_H-17 responses

has been show effective against an OVA-carrying tumor in mice[118]. Further research will show whether not only the antigen presentation capacities of CLRs but also their immune activation abilities can be harnessed to combat tumors as well as infectious diseases.

Using CLR-targeted vaccines in combination with other vaccination platforms such as nanoparticles or DNA vaccines could further strengthen vaccination strategies. CLR-targeting allows induction of specific adaptive immunity, which as an adjuvant can increase the effectivity of other vaccines that enhance antigen presentation such as DNA vaccines. Moreover, other vaccination strategies can be modulated to target CLRs. An interesting possibility would be to modulate glycosylation of viral vector-based vaccines or attenuated viruses to interact with specific CLRs and/or exclude binding to other CLRs and thereby both shape adaptive immunity and enhance antigen uptake and presentation.

Thus, despite or perhaps because of the complexity of CLR signaling, it provides a plethora of possibilities to use in host defense in vaccination design rationale, in a similar ingenious manner as successful pathogens and tumors have evolved to escape immunity.

Concluding remarks

The whole breadth of T_H responses seems to be covered by CLRs that allows for a level of diversification, which is not seen by other PRRs such as TLRs nor RLRs. Recent progress in research on CLR signaling and how this tailors efficient protective immunity to pathogens has been very important in allowing a more rational consideration behind the choice of glycoprotein/carbohydrate-conjugated antigens and adjuvants in developing vaccines. CLRs have been extensively studied as targets to enhance antigen presentation and induction of antigen-specific T cells, but now the ability of CLRs to shape adaptive immunity to pathogens should be exploited in vaccination strategies.

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Box 1 | **Dectin-1 signaling to antifungal T_H responses**

Dectin-1 recognition of fungal β-glucans induces phosphorylation of its ITAM-like motif by an unidentified Src kinase (not pictured). Subsequently, SHP2 binds to the ITAM-like motif of dectin-1 via its N-SH2 domain and phosphorylation of its ITAM-like motif by either Src or tyrosine kinase Syk facilitates recruitment of Syk to its ITAM-like motif[37]. Dimerization of dectin-1 would bring two Syk molecules together, leading to *trans*-activation of Syk, which induces the assembly of a CARD9-Bcl-10-Malt1 complex[35,36], a process that in mice[125] but not human (SI Gringhuis, TBH Geijtenbeek, unpublished observations) involves Syk-mediated phosphorylation of CARD9 by PKCδ (not pictured) and is otherwise not well defined. The CARD9-Bcl-10-Malt1 scaffold allows activation of different signaling pathways. Both the classical and non-canonical NF-κB pathways are activated, via IKKβ-mediated degradation of IκBα and NIK- and IKKα-mediated phosphorylation and proteasomal processing of NF-κB subunit p100, respectively, leading to nuclear translocation of classical p65/p50 and c-Rel/p50 and non-canonical RelB/p52 complexes[31,32,35] (SI Gringhuis, TBH Geijtenbeek, unpublished observations). Simultaneous activation of Raf-1 by dectin-1 leads to phosphorylated/acetylated p65 (see BOX 2 for details) that forms transcriptionally inactive RelB/p65 dimers. Since RelB/p52 complexes would interfere with transcription initiation of *IL1B and IL12B*, this allows for p65/p50 and c-Rel/p50 to induce expression of IL-12p40 and pro-IL-1β, as well as IL-6 and IL-23p19[31]. Syk-dependent signaling also induces nuclear translocation of IRF1 that is crucial for nucleosome remodeling at the *IL12A* promoter to enable p65/p50 binding for transcription activation[51]. Syk-mediated activation of TEC kinase triggers the integration of adaptor ASC and Malt1-pro-caspase-8 dimers into the CARD9-Bcl-10-Malt1 scaffold to form the non-canonical caspase-8 inflammasome. TEC activity is also required for cleavage and activation of caspase-8, which then processes pro-IL-1β to bioactive IL-1β[44,45]. The cooperation between Syk- and Raf-1-mediated signaling pathways leads to expression of a cytokine profile that promotes antifungal T_H1 and T_H-17 differentiation. Syk-dependent signaling also induces activation of IRF5, which promotes expression of IFNβ[40,41,126]; autocrine IFN receptor signaling then attenuates expression of T_H-17-polarizing IL-1β, while enhancing expression of T_H-17-suppressing IL-27 via transcriptional modulation of *IL27A*[41]. This signaling pathway dampens T_H-17 differentiation, possibly to prevent pathogenic hyperinflammation by T_H-17 cells.

Box 2 | **Carbohydrate-specific immunity by DC-SIGN signaling**

In immature DCs, the cytoplasmic tail of DC-SIGN is bound by adaptor protein LSP1 and the so-called Raf-1 signalosome, consisting of CNK, KSR1 and serine/threonine kinase Raf-1[56]. **a** |

Upon recognition of mannose-containing pathogens, such as fungi or mycobacteria, various proteins are recruited to the signalosome that regulate activation of Raf-1. GTPase LARG has a dual role by activating two small GTPases, Ras and RhoA[38,56,117]. Binding of GTP-Ras to Raf-1 is the initiating event in Raf-1 activation by releasing intramolecular autoinhibition[127]. Next, Raf-1 requires multiple phosphorylation. GTP-RhoA activates serine/threonine kinase Pak1, likely through Cdc42 (not pictured)[128], which phosphorylates Raf-1 at Ser338[56]. Tyr340/341 phosphorylation through an unidentified Scr kinase achieves full Raf-1 activation[56]. The pathway downstream of Raf-1 has yet to be delineated, although it does not involve the classic Raf-1 effector ERK[38], but results in Ser276 phosphorylation of NF-κB subunit p65[38]. p65/p50 complexes are activated by invading pathogens upon simultaneous TLR triggering[38]. Phospho-Ser276 recruits histone acetyltransferases CBP/p300 (not pictured) to p65, which results in acetylation of p65 on several residues[38]. The enhanced transcription rate and prolonged stability of phosphorylated/acetylated p65 enhances expression of its target genes, promoting T_H1 and T_H-17 differentiation[31,56]. **b** | Upon recognition of fucose-containing pathogens, such as parasites and *Helicobacter pylori*, the Raf-1 signalosome is displaced, while LSP1 remains bound to DC-SIGN[56] and becomes phosphorylated on Ser252 by serine/threonine kinase MK2 that is activated by almost any simultaneously triggered PRR[59]. Phosphorylated LSP1 enables assembly of a fucose-specific signalosome, consisting of serine/threonine kinase IKKε and de-ubiquitinase CYLD[59]. Close proximity enables phosphorylation of CYLD by IKKε, which deactivates CYLD and allows nuclear translocation of ubiquitinated Bcl3[59], which either positively or negatively influences transcription of target genes by binding to p50 dimers[62] (not pictured), resulting in modulation of a TLR-induced cytokine and chemokine expression profile, thereby dictating T_H2 polarization[59]. Furthermore, IKKε also phosphorylates transcription factor STAT1 on Ser708 and Ser727[8]. Once TLR-induced type I IFN triggers IFNα/βR signaling, JAK1-mediated Tyr701 phosphorylation of STAT1 leads to preferential dimerization between Ser708-phosphorylated STAT1 and STAT2. Together with IRF9, STAT1-STAT2 dimers form ISGF3 complexes that translocate into the nucleus where they enhance *IL27A* transcription that is crucial for IL-27-mediated T_{FH} differentiation[8].

Box 3 | **Dectin-2 signaling in antifungal and allergic responses**

Dectin-2 requires association with the FcRγ chain, a membrane-associated adaptor protein that contains a traditional ITAM motif, to induce intracellular signaling[73]. **a** | Upon recognition of α-1,2-mannose structures on fungi or mycobacterial ManLAM by murine dectin-2, the ITAM of the FcRγ chain is phosphorylated and bound by the two SH2 domains of SHP2, which, after

phosphorylation of its ITAM motif by Src and/or Syk kinases, facilitates binding and activation of Syk and assembly of the CARD9-Bcl-10-Malt1 scaffold (see BOX 1 for details)[37,76,78,81]. Downstream signaling leads to activation of the classical NF-κB pathway; activation of p65-containing complexes has been reported, while activation of c-Rel/p50 complexes has not been examined yet[78,81]. NF-κB activation leads to expression of the T_H-17-polarizing cytokines IL-1β, IL-6 and IL-23[76,78,80,81]. Signaling by murine dectin-2 can promote dectin-1-induced T_H1 responses, however is unable to instigate these responses by itself (not pictured)[78,80]. Furthermore, murine dectin-2 has also been shown to recognize allergens expressed by fungi (*Aspergillus fumigatus*) and house dust mites *Dermatophagoides farinae* (Df) and *D. pteronyssinus* (Dp)[83-85], although the nature of the recognized glycans remains elusive. In a Syk-dependent manner, dectin-2 recognition of allergens leads to the generation of cysteine leukotrienes (Cys-LT), which skews T_H cell differentiation towards pathogenic T_H2 responses[84,85], possibly by blocking IL-12 expression induced via other PRRs like TLRs[86]. **b** | In human, dectin-2 binding by fungi leads to FcRγ signaling via SHP2/Syk and the CARD9-Bcl-10-Malt1 scaffold[37,77]. While simultaneous triggering of dectin-1 results in activation of both classical NF-κB complexes, p65/50 and c-Rel/p50 (see BOX 1), dectin-2 engagement only leads to c-Rel/p50 activation[77], thereby further promoting expression of the T_H-17-polarizing cytokines IL-1β and IL-23[77].

Box 4 | **Mincle signaling in murine and human immune responses**

Mincle, like dectin-2 (see BOX 3), requires association with the FcRγ chain to induce intracellular signaling[87]. **a** | Murine mincle further associates with MCL, after initial binding of mycobacteria or mycobacterial cord factor (TDM, or its synthetic analogue TDB) to MCL, or has upregulated expression of mincle on murine myeloid cells[92,94,95]. MCL is also associated with the FcRγ chain[92]. It remains undefined how proximal mincle/MCL signaling proceeds, however it is likely that, like with dectin-2, SHP2 and Syk are consecutively recruited to the ITAMs of the associated FcRγ chains[37]. Mincle/MCL recognition of TDM/TDB results in Syk-dependent CARD9-Bcl-10-Malt1 scaffold formation to activate NF-κB transcriptional complexes, of which the precise composition is yet to be researched[93], and induce expression of a cytokine profile that orchestrates T_H1 and T_H-17 responses[89,92,93,101]. **b** | In human, mincle operates through FcRγ, but independent of MCL association[51,95]. Syk activation upon recognition of pathogenic *Fonseceae* species leads to CARD9-Bcl-10-Malt1 scaffold formation, but not NF-κB activation[51]. Instead, a signaling cascade via PI 3-kinase (PI3K) is initiated, however it remains elusive how the CARD9-Bcl-10-Malt1 scaffold is linked to PI3K activation[51]. PI3K activation leads to activation of the

serine/threonine kinase PKB, via phosphorylation of Thr308 and Ser473[51], which is likely mediated by kinases PDK1 and mTORC2 (not pictured), respectively[129]. PKB directly phosphorylates E3-ubiquitin ligase Mdm2 at Ser166, within the cytoplasm, which allows Mdm2 to gain nuclear entry[51,130,131]. Within the nucleus, Mdm2 associates with dectin-1-induced IRF1 (see BOX 1), which activates it ubiquitin ligase activity, hence targeting IRF1 for proteasomal degradation, which completely blocks transcriptional activation of *IL12A* via dectin-1 or TLR4, hence skewing T_H cell differentiation towards profungal T_H2 responses[51].

Table 1 | **C-type lectin receptors, pathogen recognition and T helper cell responses**

CLR	Host	Pathogen	Glycan PAMP	T_H polarizing cytokines	Adaptive response	References
DC-SIGN (CD209)			Mannose/fucose			
	Human	*Candida albicans*	Mannan	IL-12 ↑, IL-6 ↑, IL-1β ↑, IL-23 ↑	T_H1 ↑, T_H-17 ↑	31
	Human	*Fasciola hepatica*	LDNF (SP)	IL-27 ↑	T_{FH} ↑, T_H2 ↑	8
	Human	*Helicobacter pylori*	LPS-LeY	IL-12 ↓, IL-6 ↓, IL-23 ↓	T_H2 ↑	56,59,61
	Human	HIV-1	gp120	IL-12 ↑, IL-6 ↑	ND	56
	Human	*Mycobacterium tuberculosis*	ManLAM	IL-12 ↑, IL-6 ↑	ND	56
	Human	*Schistosoma mansoni*	LeX (SEA)	IL-12 ↓, IL-6 ↓, IL-23 ↓, IL-27 ↑	T_H2 ↑, T_{FH} ↑	59
	Mouse	(tissue ligands)	LeX	IL-10 ↑	T_{reg} ↑	132
Dectin-1 (CLEC7A)			β-1,3-glucan			
	Human	*Aspergillus fumigatus*		IL-23 ↑	T_H-17 ↑	133
	Human	*Candida albicans*		IL-12 ↑, IL-6 ↑, IL-23 ↑, IL-1β ↑	T_H1 ↑, T_H-17 ↑	31,44,51,77,134
	Human	*Candida lusitaniae*		IL-12 ↑, IL-6 ↑, IL-23 ↑, IL-1β ↑	T_H-17 ↑	44,77
	Human	*Candida nivariensis*		IL-12 ↑, IL-6 ↑, IL-23 ↑, IL-1β ↑	T_H-17 ↑	44,77
	Human	*Candida parapsilosis*		IL-6 ↑, IL-1β ↑	T_H-17 ↑	134
	Human	*Fonseceae monophora*		IL-12 ↑	T_H1 ↑	51
	Human	*Histoplasma capsulatum*		IL-23 ↑	T_H-17 ↑	133
	Mouse	*Aspergillus fumigatus*		IL-23 ↑	T_H-17 ↑	135
	Mouse	*Candida albicans*		IL-12 ↑, IL-23 ↑, IL-6 ↑	T_H-17 ↑	32
	Mouse	*Coccidioides immitis*		IL-12 ↑, IL-6 ↑, IL-1β ↑	T_H-17 ↑, T_H1 ↑	136
	Mouse	*Coccidioides posadasii*		ND	T_H-17 ↑	137
	Mouse	*Histoplasma capsulatum*		ND	T_H-17 ↑	137
	Mouse	*Paracoccidioides brasiliensis*		ND	T_H-17 ↑	138
	Mouse	*Trichosporon asahii*		IL-23 ↑	T_H-17 ↑	139
Dectin-2 (CLEC6A)			α-1,2-mannose			
	Human	*Candida albicans*		IL-23 ↑, IL-1β ↑	T_H-17 ↑	77

	Human	*Candida nivariensis*		IL-23 ↑, IL-1β ↑	T_H-17 ↑	77
	Mouse	*Blastomyces dermatitidis*		ND	T_H-17 ↑	137
	Mouse	*Candida albicans*		IL-12 ↑, IL-6 ↑, IL-23 ↑, IL-1β ↑	T_H-17 ↑, T_H-1 ↑	78,80,81,140
	Mouse	*Coccidioides posadasii*		ND	T_H-17 ↑	137
	Mouse	*Dermatophagoides farinae, Dermatophagoides pteronyssinus* allergens	ND	IL-23 ↑, IL-6 ↑	T_H2 ↑, T_H-17 ↑	83-85,141
	Mouse	*Fonseceae pedrosoi*		ND	T_H-17 ↑	79
	Mouse	*Histoplasma capsulatum*		ND	T_H-17 ↑	137
	Mouse	*Mycobacterium tuberculosis, M. bovis* BCG, *M. intracellulare, M. gordonae*	ManLAM	IL-12p40 ↑, IL-6 ↑	T_H-17 ↑	76
Mincle (CLEC4E)						
	Human	*Fonseceae compacta*	ND	IL-12 ↓	T_H1 ↓, T_H2 ↑	51
	Human	*Fonseceae monophora*	ND	IL-12 ↓, IL-1β ↑	T_H1 ↓, T_H2 ↑, T_H-17 ↑	41,51
	Human	*Fonseceae pedrosoi*	ND	IL-12 ↓	T_H1 ↓, T_H2 ↑	51
	Human	*Cladophialophora carrionii*	ND	IL-12 ↓	T_H1 ↓, T_H2 ↑	51
	Mouse	*Fonseceae pedrosoi*	ND	ND	T_H-17 ↓	79
	Mouse	*Malassezia pachydermatis*, *M.furfur*	Glucosyl- and mannosyl-glycolipids	IL-6 ↑	(Neutrophil infiltration)	75,88
	Mouse	*Mycobacterium bovis* BCG	TDM/TDB	ND	T_H1 ↑	97
	Mouse	*Mycobacterium tuberculosis*	TDM/TDB	IL-6 ↑, IL-1β ↑, IL-23 ↑, IL-12 ↑	T_H1 ↑, T_H-17 ↑	89,91,93,95,101

CLR, C-type lectin receptor; PAMP, pathogen-associated molecular pattern; T_H, T helper cell; T_{FH}, follicular T helper cell; SP, soluble products; SEA, soluble egg antigens; ManLAM, mannose-capped lipoarabinomannan; LDNF, fucosylated LacdiNAc; Le, Lewis antigen; TDM, trehalose-6,6-dimycolate; TDB, trehalose-6,6-dibehenate; ND, no data.

Figure 1 | **Pathogen interactions by dendritic cells dictate T helper cell differentiation. a** | Immature dendritic cells (DCs) encounter invading pathogens, resulting in uptake and processing of these microbes for antigen presentation on MHC class II molecules. Furthermore, DCs express pathogen recognition receptors (PRRs) that are triggered by pathogen-associated molecular patterns (PAMPs) resulting in innate signaling leading to gene expession and consequently DC maturation and cytokine responses. Mature DCs migrate to lymphoid tissues where they present antigens to naive $CD4^+$ T cells. TCR triggering together with secondary DC-provided signals, including cytokines/chemokines and cell-membrane receptors, then dictate pathogen-tailored T_H cell differentiation. **b** | T_H1 responses are required for defense against intracellular pathogens such as fungi, viruses and mycobacteria, and are induced by DCs that secrete IL-12. Transcription factor T-bet is the master regulator of T_H1 cell differentiation and is regulated by STAT4 that is induced by IL-12[3]. T_H2 responses are required as defense against extracellular pathogens by activating eosinophils and basophils, and by inducing antibody isotype switching to IgE in B cells[4]. T_H2 cells are characterized by the secretion of IL-4, IL-5 and IL-13 cytokines. Transcription factor GATA3 is the master regulator of T_H2 cell differentiation, which is induced by STAT6 signaling[3]. T_H-17 responses are crucial in the defense against fungi. T_H-17 cells secrete IL-17A, IL-17F and IL-22 that recruit neutrophils to sites of infection[10]. Transcription factor RORγT is the master regulator of T_H-17 cells[11]. RORγT expression is induced by STAT3 signaling via simultaneous IL-6 and IL-1β stimulation, while IL-23 plays an important role in the maintenance of T_H-17 commitment[12]. T_{FH} responses are essential for the establishment of protective long-term humoral immunity against pathogens via the generation of high-affinity antibodies[7]. T_{FH} cells produce IL-21, which is a potent cytokine known for driving antibody isotype class switch recombination and B cell proliferation[7]. Transcription factor Bcl-6 induced by STAT3 signaling acts as the master regulator of T_{FH} differentiation, including induction of IL-21 and chemokine receptor CXCR5, which is important for T_{FH} homing to B cell zones[7]. Multiple signals act in concert for the initiation of T_{FH} differentiation at the time of DC priming, with IL-6 and IL-27 having important roles in the generation of T_{FH} cells.

Figure 2 | **CLR signaling in vaccine development.** CLR targeting not only enhances antigen presentation but also offers tailoring of T_H responses in vaccinations against a pathogen or disease of choice. Different methods have been studied to target CLRs, including antigen-linked antibodies, glycosylated antigens and glycosylated particles containing antigens and possible adjuvants such as TLR ligands.

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This paper shows that dectin-1 activates Syk kinase and thereby is able to induce immunity independent of TLR signaling.

Deng, Z. *et al.* Tyrosine phosphatase SHP-2 mediates C-type lectin receptor-induced activation of the kinase Syk and anti-fungal TH17 responses. *Nat. Immunol.* **16**, 642-652 (2015).
This paper shows that tyrosine phosphatase SHP-2 acts as a scaffold for recruitment of Syk to dectin-1 or to the adaptor FcRγ chain, thereby controlling immunity induced by different CLRs.

Gringhuis, S.I. *et al.* Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1beta via a noncanonical caspase-8 inflammasome. *Nat. Immunol.* **13**, 246-254 (2012).
This paper identifies a caspase-8-containing complex induced by fungal and mycobacterial infections that leads to direct processing of IL-1β, independently of caspase-1.

Barrett, N.A. *et al.* Dectin-2 mediates Th2 immunity through the generation of cysteinyl leukotrienes. *J. Exp. Med.* **208**, 593-604 (2011).
This study shows that dectin-2 contributes to allergic T_H2 responses by inducing the production of cysteinyl leukotrienes.

Yamasaki, S. *et al.* Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat. Immunol.* **9**, 1179-1188 (2008).
This study identifies the ligand for Mincle and shows the importance of this receptor as sensor of damaged cells.

Werninghaus, K. *et al.* Adjuvanticity of a synthetic cord factor analogue for subunit Mycobacterium tuberculosis vaccination requires FcRgamma-Syk-Card9-dependent innate immune activation. *J. Exp. Med.* **206**, 89-97 (2009).
This study describes an important role for FcRγ signaling in the induction of immune responses to mycobacterial cord factor.

LeibundGut-Landmann, S., Osorio, F., Brown, G.D., Reis e Sousa & C. Stimulation of dendritic cells via the dectin-1/Syk pathway allows priming of cytotoxic T-cell responses. *Blood* **112**, 4971-4980 (2008).
This study shows how dectin-1 can be targeted to activate cytotoxic T cell responses.

Glossary terms

PAMPs

Pathogen-associated molecular patterns (PAMPs) are small conserved molecular motifs found on microbes such as lipopolysaccharides (LPS), carbohydrate moieties, dsRNA and unmethylated DNA motifs.

T helper (T_H) cells

T_H cells secrete distinct patterns of cytokines after activation, which occurs through ligation of their T-cell receptors with their cognate ligands (peptide–MHC complexes), together with recognition of the appropriate co-stimulatory molecules. Naive T_H cells differentiate into T_H1, T_H2, T_H-17, T_{FH}, T_H9, or T_H22 cells, depending on the cytokines and co-stimulatory molecules presented to them by antigen-presenting cells, providing tailored responses to combat distinct invading pathogens.

C-type lectin receptors

C-type lectin receptors (CLRs) recognize carbohydrates expressed by microbes as well as endogenous glycoproteins. Some CLRs function as pattern recognition receptors, whereas others function as adhesion receptors. CLR binding to microbes on antigen-presenting cells leads to internalization and antigen processing, while, additionally, some CLRs efficiently induce signaling pathways to induce or modulate T_H cell differentiation.

Inflammasome

Inflammasome complexes contain an inflammasome sensor molecule (such as NLRP3), the adaptor protein ASC and caspase-1. The term 'non-canonical inflammasome' is coined to describe an inflammasome-like complex that does not conform to the three 'canonical' components of the inflammasome, however, like canonical inflammasomes, processes immature pro-IL-1β protein to generate bio-active IL-1β for secretion. Two non-canonical inflammasomes have been described so far: one containing MALT1, ASC and caspase-8 (termed the non-canonical caspase-8 inflammasome) that is triggered upon dectin-1 engagement, and a caspase 11-activating platform that has not yet been fully described.

DC-SIGN signalosome

DC-SIGN signaling is controlled by distinct multi-protein ‘signalosome’ complexes that interact with LSP1 that is bound to the cytoplasmic domain of DC-SIGN. Mannose signaling results in activation of serine/threonine kianse Raf-1 that is present together with CNK and KSR1 in the ‘Raf-1 signalosome’. Fucose binding to DC-SIGN leads to disassociation of the

Raf-1 signalosome, instead recruiting serine/threonine kinase IKKε and de-ubiquitinase CYLD to LSP1. Both signalosomes are paramount in transducing signals from extracellularly bound ligands to the nucleus to modulate cytokine transcription programs.

Online summary

- C-type lectin receptors (CLRs) are efficient pattern recognition receptors (PRRs) that interact with pathogens via carbohydrate structures, leading to enhanced antigen presentation as well as modulation of T helper (T_H) cell differentiation, collectively inducing pathogen-tailored adaptive immune responses.
- Cross-talk between innate signaling by CLRs and other PRRs as well as other receptors such as IFN receptor enhances the diversity of T_H responses to pathogens.
- Dectin-1 engagement by fungi induces different intracellular signaling pathways that cooperate to induce efficient T_H1 and T_H-17 responses, which are crucial to antifungal immunity.
- DC-SIGN induces carbohydrate-specific signaling to distinct pathogens; mannose recognition on intracellular pathogens such as fungi, viruses or mycobacteria leads to protective T_H1 responses together with concomitant TLR signaling, whereas parasitic fucose-containing ligands induce both T_H2 and T_{FH} responses, necessary for humoral immunity, dependent on TLR and IFN receptor crosstalk.
- CLR triggering also contributes to pathogenic disorders; dectin-2 engagement by allergens originating from fungi or house dust mite leads to allergic T_H2 responses, while mincle triggering by pathogenic fungi from *Fonseceae* species represses T_H1 responses, thereby promoting fungal dissemination.
- CLRs have been used previously in vaccination strategies for their antigen processing capacity. The advanced knowledge of the signaling pathways by which CLRs direct adaptive immunity now provides a powerful tool to add a novel level of sophistication to the design of vaccines. Especially the very specific T_{FH} responses that are induced by CLRs such as DC-SIGN and CLEC9A could greatly improve generation of broadly neutralizing antibodies against viruses such as HIV-1.

Author biographies

Teunis Geijtenbeek is professor in Molecular and Cellular Immunology at the Academic Medical Center, Amsterdam, the Netherlands, and head of the Department of Experimental Immunology. He was previously affiliated with the Department of Molecular Cell Biology and Immunology at the VU University Medical Center in Amsterdam. His research has focused on C-type lectin receptors, host-pathogen interactions and their function in host defense for the past 16 years.

Sonja Gringhuis is an associate professor at the Academic Medical Center, and principal investigator of the Host Defense group, together with Teunis Geijtenbeek. She received her Ph.D. in Molecular Biology from the University of Groningen (The Netherlands). Her research is driven by an innate desire to solve the puzzles that the signaling pathways behind immunological responses present. For the past 11 years, her research has focused on the molecular mechanisms of C-type lectin signaling and the effects on adaptive immunity.

Table of contents summary

Here the authors discuss the role of C-type lectin receptor signaling pathways in the control of T helper cell differentiation and how this signaling can be harnessed for vaccination strategies.

Figure 1

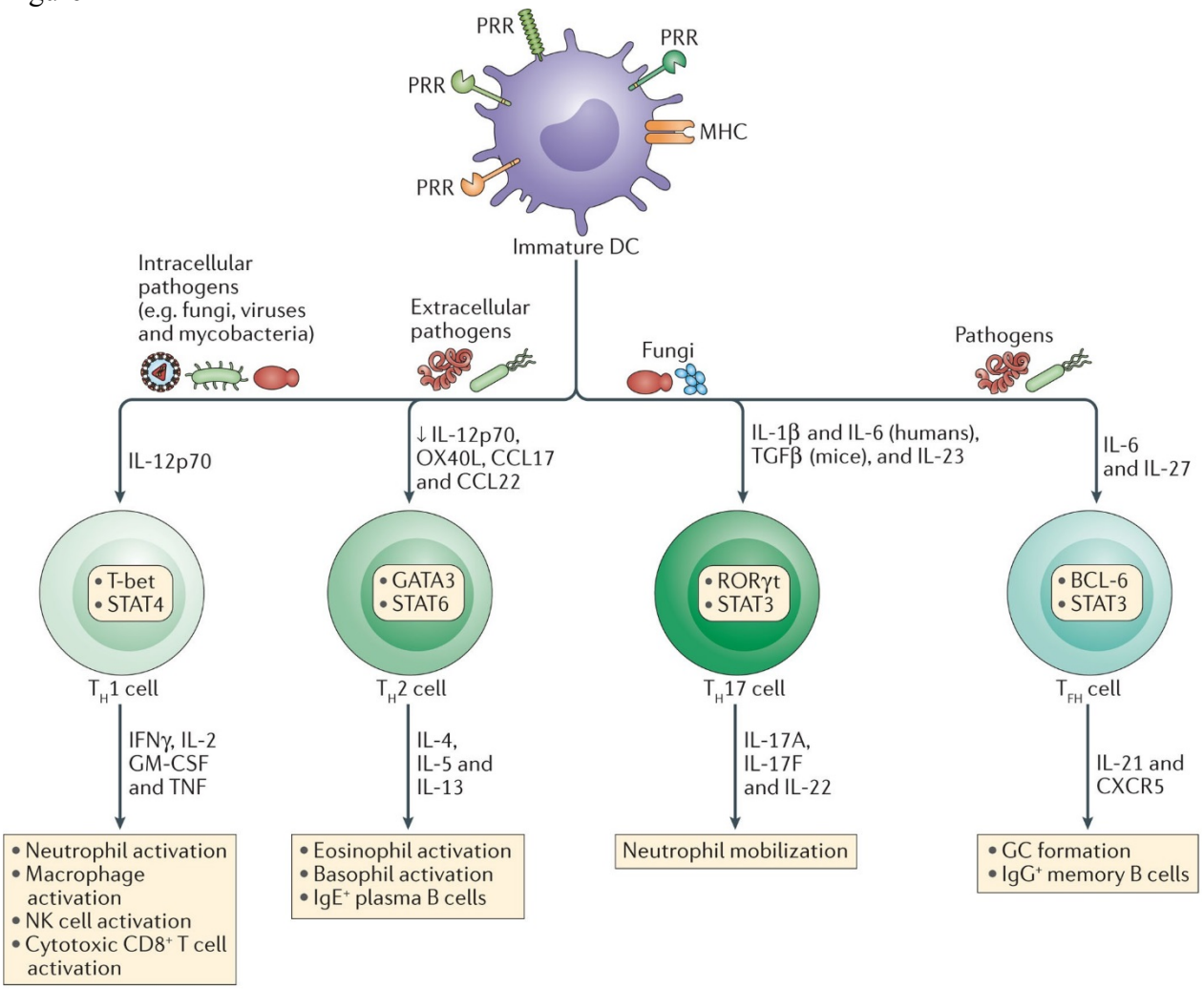
PRR
PRR
PRR
PRR
MHC
Immature DC
Intracellular pathogens (e.g. fungi, viruses and mycobacteria)
Extracellular pathogens
Fungi
Pathogens
IL-12p70
↓ IL-12p70, OX40L, CCL17 and CCL22
IL-1β and IL-6 (humans), TGFβ (mice), and IL-23
IL-6 and IL-27
• T-bet • STAT4
• GATA3 • STAT6
• RORγt • STAT3
• BCL-6 • STAT3
T_H1 cell
T_H2 cell
T_H17 cell
T_{FH} cell
IFNγ, IL-2 GM-CSF and TNF
IL-4, IL-5 and IL-13
IL-17A, IL-17F and IL-22
IL-21 and CXCR5
• Neutrophil activation • Macrophage activation • NK cell activation • Cytotoxic CD8⁺ T cell activation
• Eosinophil activation • Basophil activation • IgE⁺ plasma B cells
Neutrophil mobilization
• GC formation • IgG⁺ memory B cells
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Figure 2

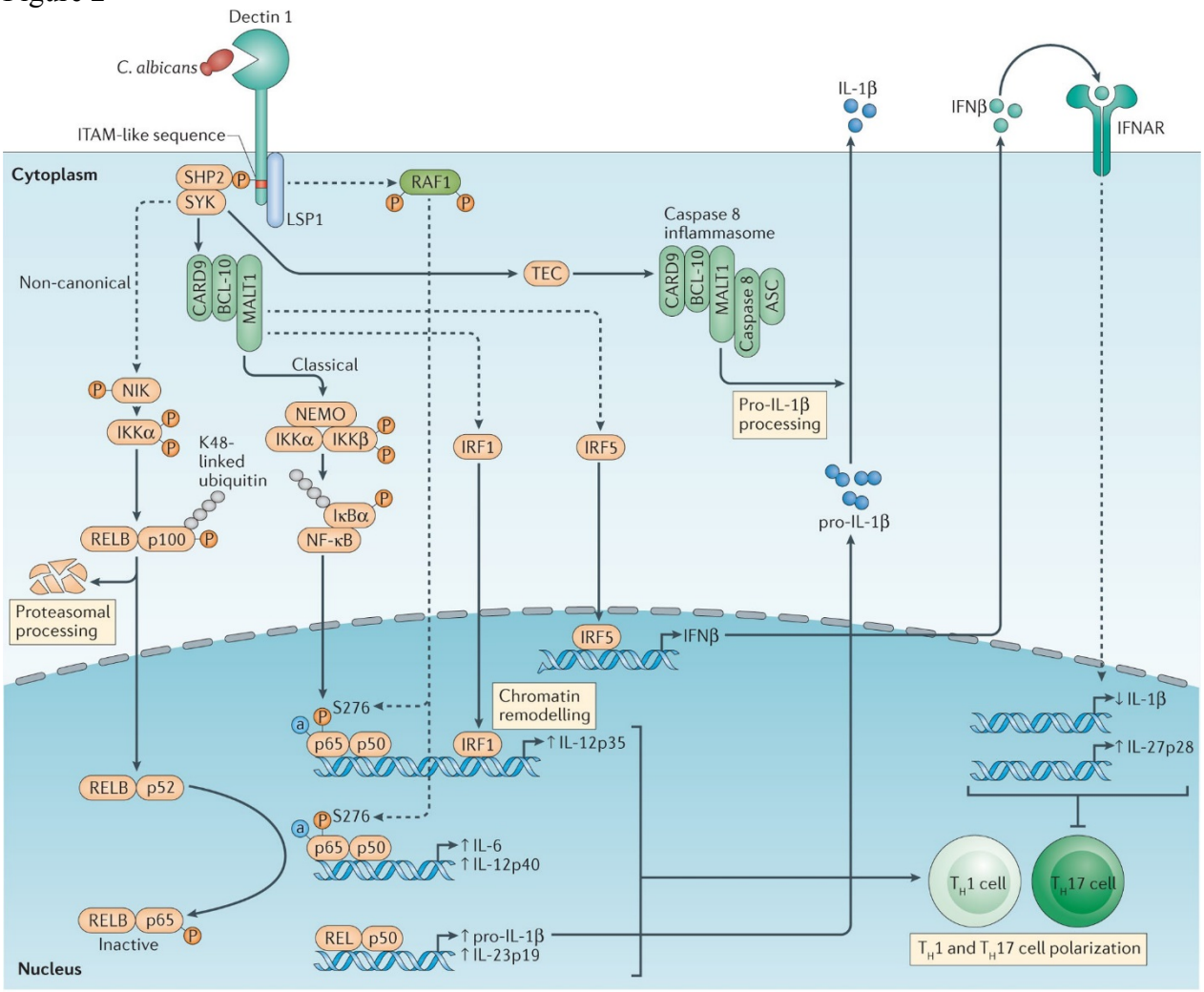

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Figure 3

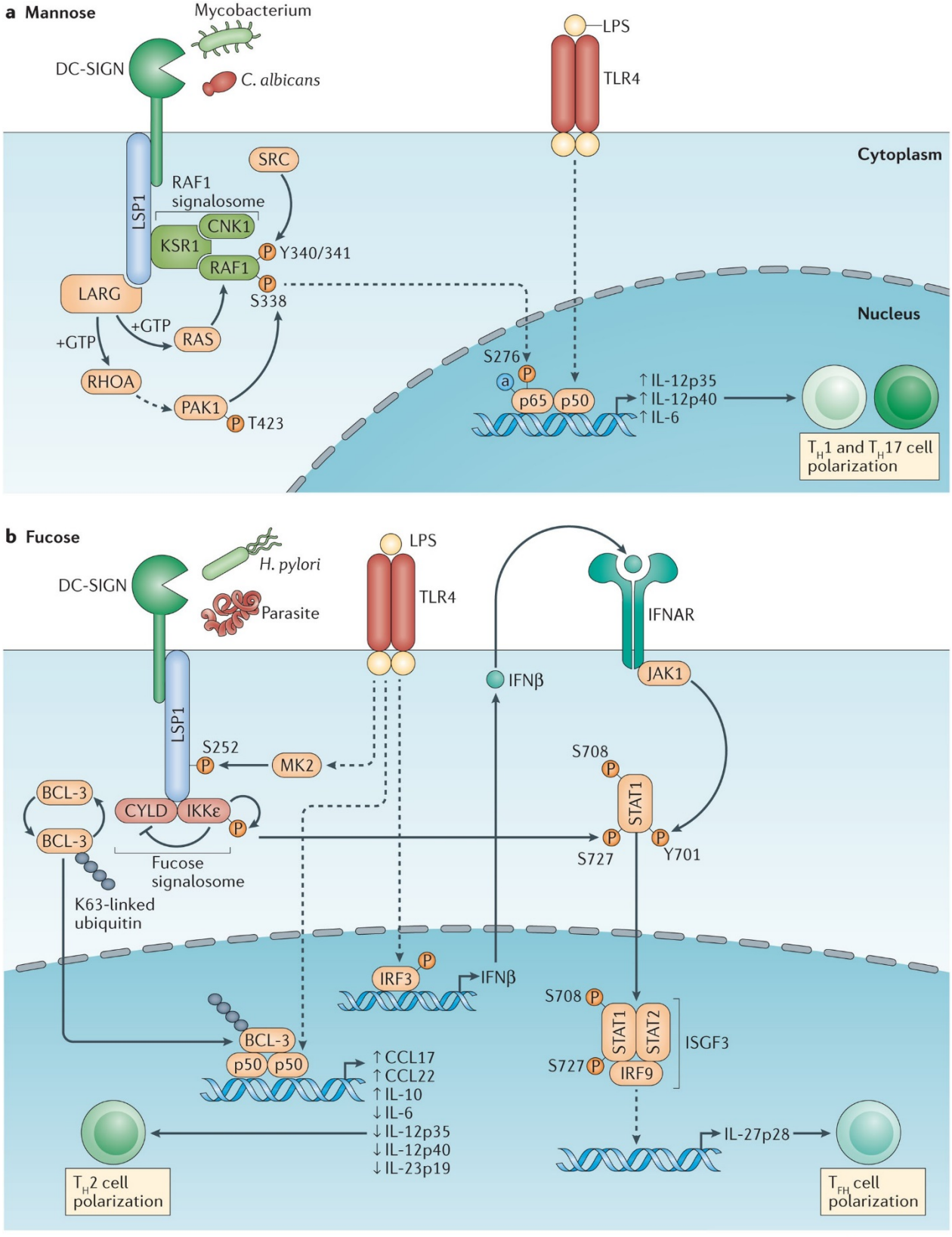
a Mannose
Mycobacterium
DC-SIGN
C. albicans
LPS
TLR4
Cytoplasm
SRC
RAF1 signalosome
LSP1
CNK1
KSR1
RAF1
Y340/341
S338
LARG
+GTP
RAS
RHOA
PAK1
T423
Nucleus
S276
a
p65
p50
↑ IL-12p35
↑ IL-12p40
↑ IL-6
T_H1 and T_H17 cell polarization
b Fucose
H. pylori
Parasite
DC-SIGN
LPS
TLR4
IFNAR
IFNβ
JAK1
LSP1
S252
MK2
S708
STAT1
BCL-3
CYLD
IKKε
S727
Y701
Fucose signalosome
K63-linked ubiquitin
IRF3
IFNβ
S708
STAT1
STAT2
ISGF3
S727
IRF9
BCL-3
p50
p50
↑ CCL17
↑ CCL22
↑ IL-10
↓ IL-6
↓ IL-12p35
↓ IL-12p40
↓ IL-23p19
IL-27p28
T_H2 cell polarization
T_FH cell polarization
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Figure 4

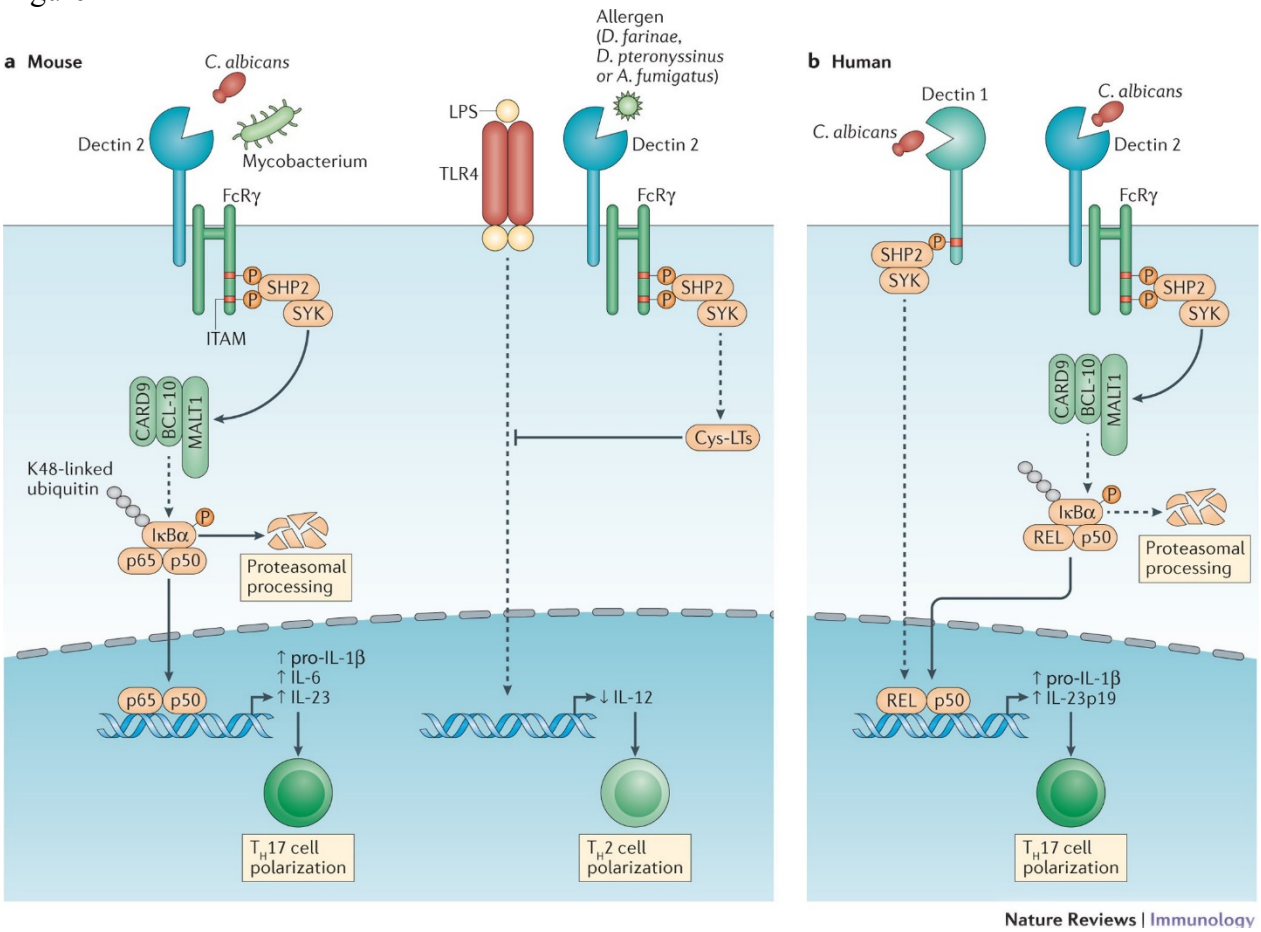
a Mouse
C. albicans
Dectin 2
Mycobacterium
FcRγ
P
P
SHP2
SYK
ITAM
CARD9
BCL-10
MALT1
K48-linked
ubiquitin
P
IκBα
p65
p50
Proteasomal
processing
↑ pro-IL-1β
↑ IL-6
↑ IL-23
p65
p50
T$_H$17 cell
polarization
LPS
TLR4
Allergen
(D. farinae,
D. pteronyssinus
or A. fumigatus)
Dectin 2
FcRγ
P
P
SHP2
SYK
Cys-LTs
↓ IL-12
T$_H$2 cell
polarization
b Human
Dectin 1
C. albicans
SHP2
SYK
P
C. albicans
Dectin 2
FcRγ
P
P
SHP2
SYK
CARD9
BCL-10
MALT1
P
IκBα
REL
p50
Proteasomal
processing
REL
p50
↑ pro-IL-1β
↑ IL-23p19
T$_H$17 cell
polarization
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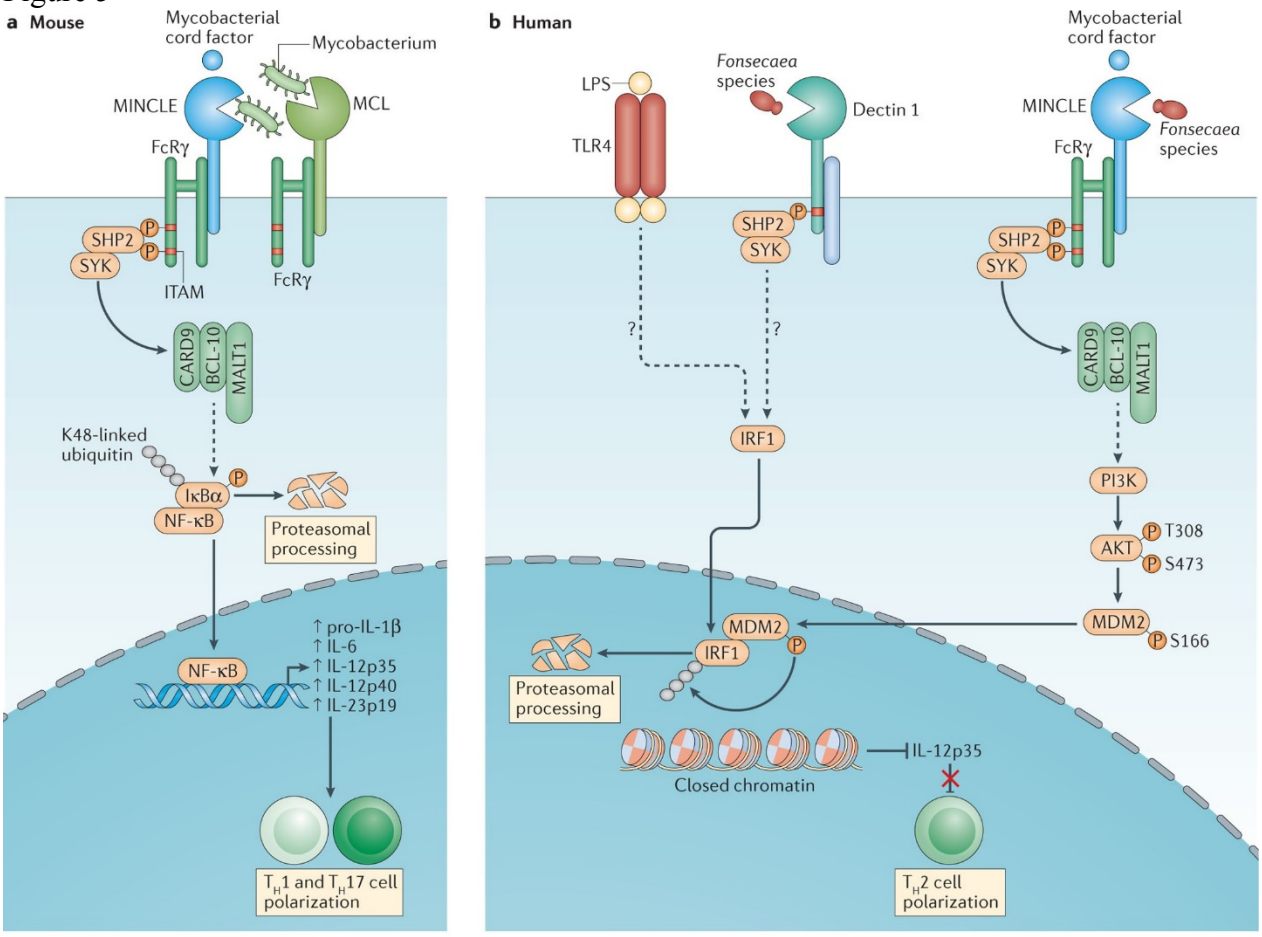
Figure 5


a Mouse
Mycobacterial cord factor
Mycobacterium
MINCLE
MCL
FcRγ
SHP2
SYK
ITAM
FcRγ
CARD9
BCL-10
MALT1
K48-linked ubiquitin
IκBα
NF-κB
Proteasomal processing
NF-κB
↑ pro-IL-1β
↑ IL-6
↑ IL-12p35
↑ IL-12p40
↑ IL-23p19
TH1 and TH17 cell polarization
b Human
LPS
TLR4
Fonsecaea species
Dectin 1
SHP2
SYK
?
?
IRF1
MDM2
IRF1
Proteasomal processing
Closed chromatin
IL-12p35
TH2 cell polarization
Mycobacterial cord factor
MINCLE
Fonsecaea species
FcRγ
SHP2
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CARD9
BCL-10
MALT1
PI3K
AKT
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S473
MDM2
S166
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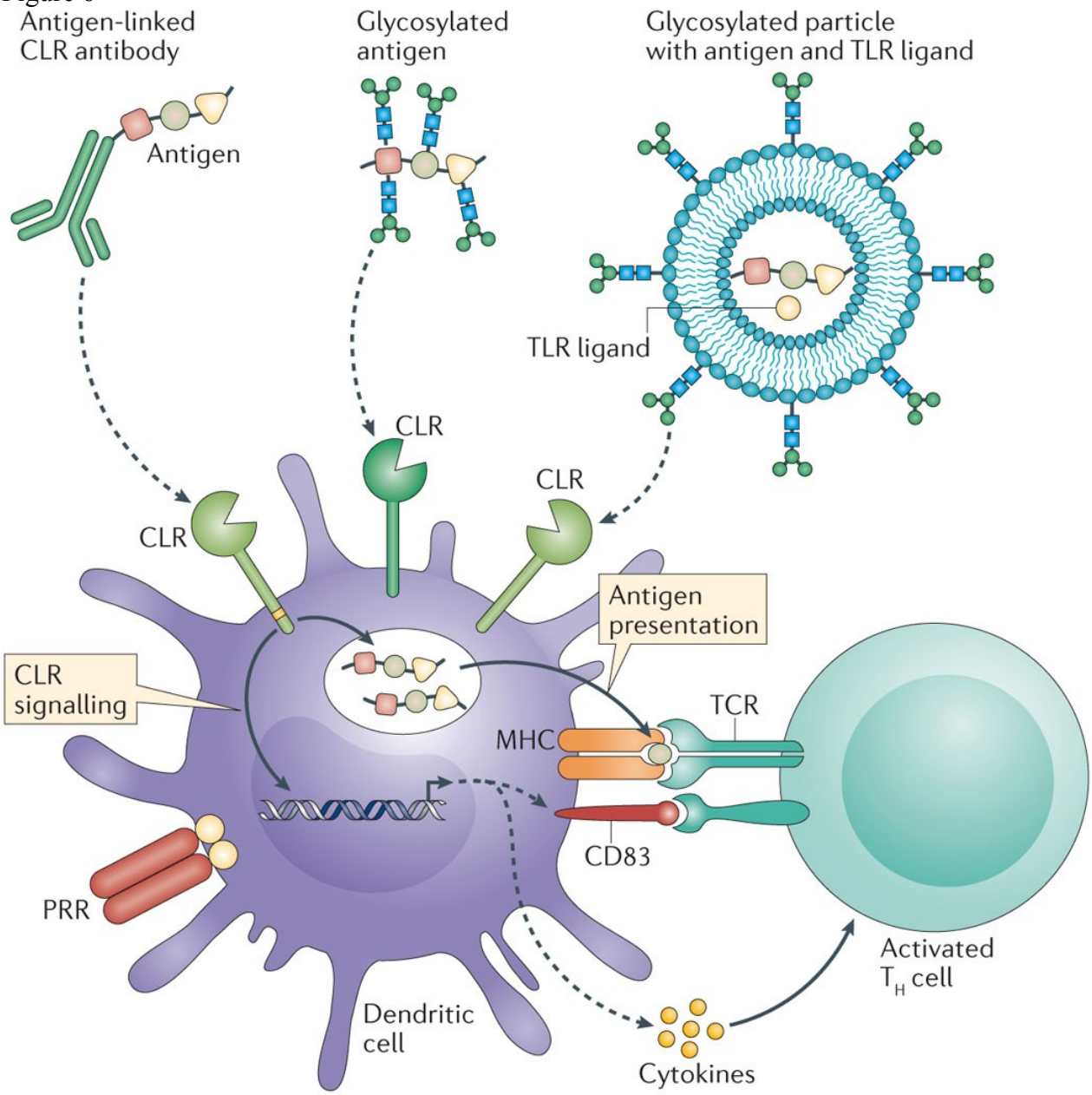
Figure 6


Antigen-linked CLR antibody
Glycosylated antigen
Glycosylated particle with antigen and TLR ligand
Antigen
TLR ligand
CLR
CLR
CLR
Antigen presentation
CLR signalling
TCR
MHC
CD83
PRR
Activated T_H cell
Dendritic cell
Cytokines
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