Chapter 1
C-Type Lectin Receptors in Antifungal Immunity

Christina Nikolakopoulou, Janet A. Willment and Gordon D. Brown

Abstract Most fungal species are harmless to humans and some exist as commensals on mucocutaneous surfaces. Yet many fungi are opportunistic pathogens, causing life-threatening invasive infections when the immune system becomes compromised. The fungal cell wall contains conserved pathogen-associated molecular patterns (PAMPs), which allow the immune system to distinguish between self (endogenous molecular patterns) and foreign material. Sensing of invasive microbial pathogens is achieved through recognition of PAMPs by pattern recognition receptors (PRRs). One of the predominant fungal-sensing PRRs is the C-type lectin receptor (CLR) family. These receptors bind to structures present on the fungal cell wall, eliciting various innate immune responses as well as shaping adaptive immunity. In this chapter, we specifically focus on the four major human fungal pathogens, *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Pneumocystis jirovecii*, reviewing our current understanding of the CLRs that are involved in their recognition and protection of the host.

Keywords Pathogenic fungi · Antifungal immunity · PRRs · CLRs

1.1 Introduction

Fungi are eukaryotic organisms that include yeasts, moulds and mushrooms. The fungal kingdom is large and distinct from animals and plants, with over one million

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fungal species mainly found on organic substrates, such as plant debris and soil (Mora et al. 2011). It has been estimated that around 600 fungal species can cause disease in humans (Brown et al. 2012). Most human-associated fungi are found as commensals on mucocutaneous surfaces, such as *Candida* and *Malassezia* species and do not cause any disease in healthy individuals (Havlickova et al. 2008). Fungal pathogens rarely cause disease in immunocompetent people, with the most frequent infections being onychomycosis, keratitis (corneal infection that can lead to blindness) and dermatophytosis (infection of the skin) and in rare occasions invasive infections (Brown et al. 2012). However, commensal fungi and many environmental fungi can become opportunistic pathogens when there is a disruption in physical barriers, when the immune system of the host is compromised, or in times of fungal dysbiosis (Brown 2011; Iliev and Leonardi 2017). The incidence of invasive fungal infections (IFI) has increased significantly over the last 30 years mainly because of the HIV/AIDS pandemic and invasive and immunosuppressive modern medical treatments (Erwig and Gow 2016). Recent estimates indicate more people die globally from fungal infections than malaria and about the same number die from tuberculosis (Brown et al. 2012); yet significantly less research is conducted on fungal infections. Even though in the UK, between 1997 and 2010, £2.6 billion was invested in research on all infectious diseases (Head et al. 2013), only 2% of this was put towards medical mycology research (Head et al. 2014). As a result, many people are unaware of their huge impact and most of the times fungal diseases are undiagnosed. Some significant problems facing the field are the lack of appropriate diagnostics and vaccines. Although antifungal drugs are available, they suffer from toxicity, rising resistance and other complications. About 1.6 million people die each year because of lethal IFI (https://www.gaffi.org/why/fungal-disease-frequency/) (Brown et al. 2012). In the developed world, the mortality rate due to common life-threatening fungal infections, even in non-AIDS patients, often exceeds 50% despite treatment (https://www.gaffi.org/why/fungal-disease-frequency/).

Fungal pathogens are primarily recognised by a family of pattern recognition receptors (PRRs), known as C-type lectin receptors (CLRs). In this chapter, we will focus on the CLRs involved in the recognition of the four fungal pathogens with the highest disease burden; *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Pneumocystis jirovecii*. These cause invasive candidiasis, invasive and chronic pulmonary aspergillosis (CPA), cryptococcal meningitis and pneumocystis pneumonia, respectively (https://www.gaffi.org/why/fungal-disease-frequency/). A brief introduction to the fungal cell wall and the major CLRs recognising these structures will be presented, and then the roles of these receptors in the control of each pathogen will be discussed in detail.

1.2 Structure of the Fungal Cell Wall

The cell wall of fungal pathogens is a perfect target for our immune system to separate “self” from “non-self” as it consists of proteins and polysaccharides that

are not present in mammalian cells. However, recognition of fungi is complicated by their ability to adopt different morphologies, such as yeasts, hyphae, encapsulated cells and enlarged cells, making it hard for phagocytic cells to detect and kill these pathogens. Fungi become a "moving target" for the immune system in response to metabolic adaptation during infection. For example, *C. albicans* changes its cell wall components upon exposure to the host metabolite lactate, preventing immune recognition (Ballou et al. 2016). As the fungal cell wall has been extensively reviewed elsewhere (Erwig and Gow 2016), the following paragraphs will provide a brief context for our review.

Fungi have an inner and an outer cell wall that are made of different components and the proportion of these components varies in the different morphologies and species (see following sections for more details). Approximately 90% of the cell wall is comprised of carbohydrates and the remainder are proteins. The inner cell wall of almost all fungi is made up of β-glucans and chitin (Fig. 1.1). In the case of

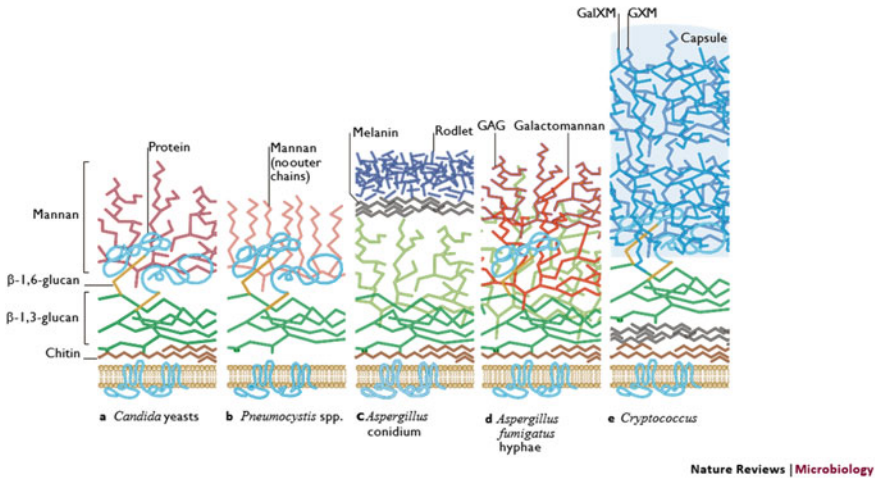

Fig. 1.1 Fungal cell wall composition and recognition by phagocytes. Figure and legend from Erwig and Gow (2016) with permission. The cell wall structures of selected fungal cells are depicted schematically showing the various layers. The core inner skeletal layer is comprised of the highly pro-inflammatory β-1,3-glucan, β-1,6-glucan and chitin. **a** In the cell walls of *Candida* species, the outer layer comprises mainly pro-inflammatory O-linked mannans, N-linked mannans and phosphorylated mannans. **b** Recent genomics analysis suggests that *Pneumocystis* spp. lack the enzymes that are required to synthesize chitin and the outer chain N-mannans, but have an outer wall with core N-mannan and O-mannan proteins. **c**, **d** The conidial spore wall of *Aspergillus* spp. consists of an immunologically inert outer hydrophobin rodlet layer and includes an inner melanin layer (part **c**), whereas the hyphae of *Aspergillus fumigatus* have a typical inner cell wall composition but have α-1,3-glucan, galactomannan and galactosaminoglycan (GAG) in the outer cell wall, along with a reduced amount of glycoprotein compared with the walls of *Candida* species (part **d**). **e** The cell wall of *Cryptococcus* is surrounded by a thick capsule of glucuronoxylomannan (GXM) and galactoxylomannan (GalXM), which is sloughed off in large quantities and can inhibit phagocyte function, and the inner wall has a layer of melanin

P. jirovecii, though, it is still debatable whether chitin is present in the inner cell wall (Skalski et al. 2015; Ma et al. 2016).

The outer cell wall layer of *C. albicans* contains mainly O- and N-linked mannans that form glycoproteins through their covalent association with proteins (Erwig and Gow 2016). The inner cell wall is more dense than the outer cell wall, primarily due to the covalent attachment of chitin, galactomannan and other proteins to β-glucans (Van De Veerdonk et al. 2017) (Fig. 1.1a). *C. albicans* can transit from the yeast to hyphal form, modifying the composition of the cell wall with changes in protein content, increased chitin, modified glucan structures (closed β-glucan linked structures) and glycosylation (Chaffin et al. 1998; Heilmann et al. 2011; Lowman et al. 2014).

During its life cycle, *A. fumigatus* germinates from conidia to form hyphae, which changes the composition of the cell wall. As a result, different PAMPs are exposed (Fig. 1.1c, d). Specifically, the conidia cell wall contains α-1,3-glucan, 1,8-dihydroxynaphthalene-(DHN) melanin (synthesised from the pentaketide pathway) and a rodlet layer made of hydrophobins (highly organised hydrophobic proteins). This rodlet layer coats the conidial outer cell wall (Fig. 1.1c) (Van De Veerdonk et al. 2017). Upon hyphae formation, the rodlet layer is removed and the melanin layers are lost completely. Hyphae then form an extracellular matrix (ECM) that contains α-1,3-glucans, galactomannan, galactosaminogalactan (GAG) and proteins that can enhance disease (Fig. 1.1d).

A characteristic feature of *C. neoformans* cell wall that is not found in *C. albicans* or *A. fumigatus* cell wall is a thick gelatinous capsule. This surrounds the outer wall of *C. neoformans* and is composed of polysaccharides including glucuronoxylomannan (GXM) and galactoxylomannan (GalXM) (Fig. 1.1e). The capsule masks cell wall β-glucan from immune recognition (see below), inhibiting the production of pro-inflammatory cytokines and protects the pathogen from phagocytosis, due to its negative charge, and from antibody and complement depletion (Buchanan and Murphy 1998). The inner wall of *C. neoformans* contains 3,4-dihydroxyphenylalanine (DOPA) melanin, which unlike *A. fumigatus* 1,8-DHN melanin, is formed by laccase and protects *C. neoformans* against the host immune response through its antioxidant ability (Casadevall et al. 2000). In the cell wall of *C. neoformans*, melanin is accumulated in melanin granules that consist of fungal melanosomes. These granules are associated with proteins that contribute to the generation of melanin as well as its structure. Melanisation of *C. neoformans* is thought to be similar to mammalian melanisation; in both systems melanin synthesis occurs inside specialised vesicles, the melanosomes, that provide protection to the host cell from toxic reaction intermediates (Camacho et al. 2019). *C. neoformans* can form titan cells during infection that have a gigantic size of up to 100 μm, in contrast to the size of typical cells, which are 5–7 μm in cell diameter (Zaragoza et al. 2010). These cells expose different PAMPs including altered chitin content in the cell wall (Mukaremera et al. 2018).

Lastly, the outer cell wall of *P. jirovecii* contains highly mannosylated (N-mannan and O-mannan) glycoproteins that mask β-glucan. Unlike *C. albicans* and *A. fumigatus*, however, the outer cell wall of *P. jirovecii* contains a heavily mannosylated glycoprotein A (gpA or MSG), but not the outer chain of N-mannans (Fig. 1.1b)

(Ma et al. 2016). The life cycle of *Pneumocystis* is uniquely adapted to growth in its host having lost the ability to grow outside the lung, and it is difficult to grow this fungus in vitro. Inside the lung, *Pneumocystis* undergoes three life cycle stages; cyst, precystic form and trophic form, which differ in size and shape. The precyst and trophic form are similar in cell wall composition (Chabé et al. 2011) and lack β-glucan (Nollstadt et al. 1994).

1.3 Pattern Recognition Receptors (PRRs)

The first line of defence against fungal pathogens is provided by the skin, mucosal surfaces and antimicrobial peptides (AMPs). AMPs are secreted by epithelial cells or produced in secretions and are active against many fungi, including *C. albicans*, *A. fumigatus* and *C. neoformans* (Schop 2007; Brown 2011). However, this protection is lost when these physical barriers are breached, and the pathogen invades deeper tissues. This invasion is sensed by the innate immune system which becomes activated, promoting the recognition and killing of the invading organisms. Innate recognition occurs through germline-encoded pattern recognition receptors (PRRs), which are expressed in nearly all cell types and recognise pathogen-associated molecular patterns (PAMPs) (Janeway 1992). As mentioned above, the cell wall contains many fungal PAMPs, which are recognised by PRRs via two mechanisms. The first is indirect recognition, where the fungal pathogens are opsonised by soluble PRRs, e.g. the complement receptor (CR3), enabling recognition through opsonic receptors. The second mechanism is direct recognition, where cell surface PRRs bind directly to the pathogen (Brown 2011) (Fig. 1.2).

All cells express PRRs. Epithelial and endothelial cell-expressed PRRs play an important role in antimicrobial immunity by stimulating the release of AMPs and inflammatory cytokines. For example, oral epithelial cells contribute to the host defence against oropharyngeal candidiasis (OPC) as they can sense *C. albicans* and upon invasion, they secrete AMPs and pro-inflammatory cytokines (Swidergall and Filler 2017). Another example is the expression of the newly identified CLR, Melanin sensing C-type lectin receptor (MelLec, which is discussed further below), which is expressed predominantly by endothelial cells in mice, and recognises *A. fumigatus* 1,8-DHN melanin (Stappers et al. 2018). Neutrophils, macrophages and other phagocytic cells use PRRs to take up and kill pathogens, as well as stimulating pro-inflammatory responses (Fig. 1.2). Following uptake and killing, antigen-presenting cells (APCs), such as dendritic cells (DCs) process antigenic fragments on major histocompatibility complex (MHC) class I or II molecules for presentation and activation of naïve T cells, and ultimately induce an adaptive immune response. The control of fungal infections is primarily reliant on Th1 and Th17 adaptive immunity (Brown 2011).

There are four families of PRRs that recognise microbial pathogens; Toll-like receptor (TLR), Nod-like receptor (NLR), RIG-I-like receptor (RLR) and C-type

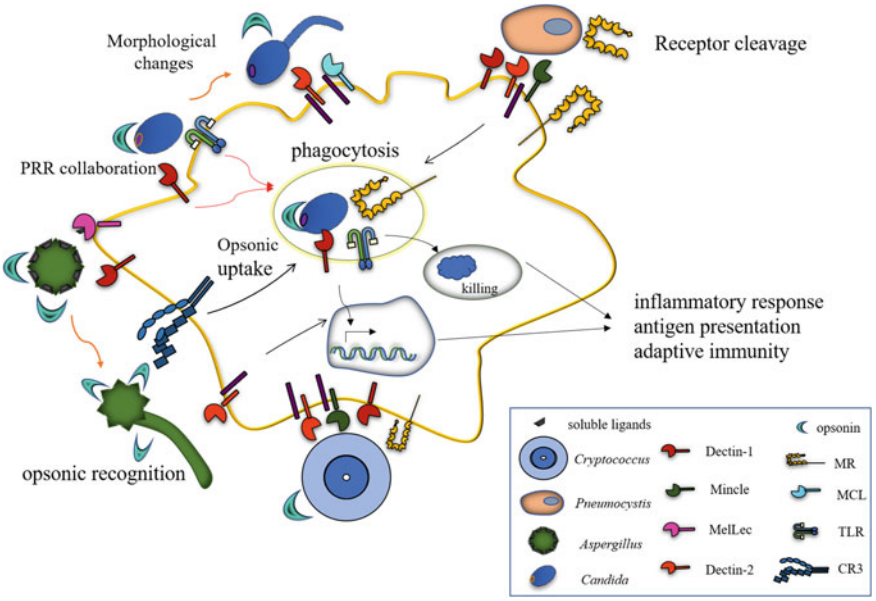

Fig. 1.2 Recognition of fungal pathogens by pattern recognition receptors (PRRs). Recognition of fungi takes place via numerous mechanisms, both direct and indirect, by cells of the innate immune system such as macrophages and neutrophils, and non-immune cells such as epithelial and endothelial cells. Indirect mechanisms of recognition are by Fc Receptors or complement receptors, such as CR3 (shown here as a heterodimer of CD11b and CD18), of fungi coated by serum opsonins (green crescent), such as antibodies and complement, respectively. Soluble PPRs, such as collectins, surfactant proteins-A and D and mannose-binding lectins can act as opsonins. Direct mechanisms of recognition by membrane bound PPRs, such as mannose receptor (MR), Dectin-1, Dectin-2, macrophage C-type lectin (MCL), Melanin sensing C-type lectin receptor (MelLec) and Toll-like receptors (TLRs) interact with fungi on the cell surface and some are recruited to the phagosome. Intracellular recognition of fungi occurs, both in the cytoplasm and vacuoles by Nod-like receptors (NLRs) and TLRs, respectively (not shown). Collaboration between various PRRs, such as Dectin-1 and the TLRs (red arrows) or between CLRs can alter the antifungal response. Depending on the cell type, recognition and uptake induces the production of inflammatory mediators and release of reactive oxygen species and antifungal peptides (black arrows). Fungal antigen presentation and cytokine production by antigen presenting cells, such as dendritic cells, shapes the adaptive immune response. Fungi undergo morphological changes (orange arrows) which alter their PAMP exposure (e.g. *Candida* masks its β-glucan exposure) and consequently the range of PRRs that recognise them differs. Fungi, such as *Pneumocystis*, have been shown to induce cleavage of PRRs, e.g. MR which binds to the fungus and alters the immune response. Fungal derived cell wall components, such as 1,8-dihydroxynaphthalene (black trapezium), which forms the melanin layer in *Aspergillus* conidia, can be recognised by the endothelial membrane bound receptor MelLec, but is shed once swelling and germination occurs

lectin receptor (CLR) families. We will focus here on the CLRs, which are the main PRRs involved in antifungal immunity.

1.4 C-Type Lectin Receptors (CLRs)

The CLRs are a diverse family of receptors that contain one or more C-type lectin-like domain (CTLD). These proteins can be soluble or transmembrane bound and recognise most human fungal pathogens through conserved motifs found in the CTLD. These motifs include QPD (Gln–Pro–Asp) and EPN (Glu–Pro–Asn), which specifically recognise galactose-type and mannose-type carbohydrates, respectively (Weis et al. 1998; Zelensky and Gready 2005). Soluble CLRs including the collectins, surfactant protein A (SP-A), SP-D, mannose-binding lectin (MBP) and collectin kidney protein 1 (CL-K1; also known as collectin 11), can act as opsonins and antimicrobial proteins (Brown et al. 2018). Here, we will focus only on transmembrane receptors. Initially, it was thought that CLRs bound only to carbohydrates in a Ca^{2+}-dependent manner, hence the name of this class of receptors. However, it is now known that many CLRs not only bind non-carbohydrates (e.g. lipids and proteins) via CTLDs but can also bind ligands in a Ca^{2+}-independent fashion (e.g. β-glucan). The CLRs have been separated into 17 groups based on their structure and phylogeny (Zelensky and Gready 2005). See http://www.imperial.ac.uk/research/animallectins/ctld/mammals/humanvmousedata.html for the full list of CTLD receptor groups.

CLR members of the group II, V and VI receptors (Fig. 1.3) are predominantly expressed on myeloid cells and are involved in antifungal immunity and will be discussed here. The group VI CLRs are type I transmembrane receptors with

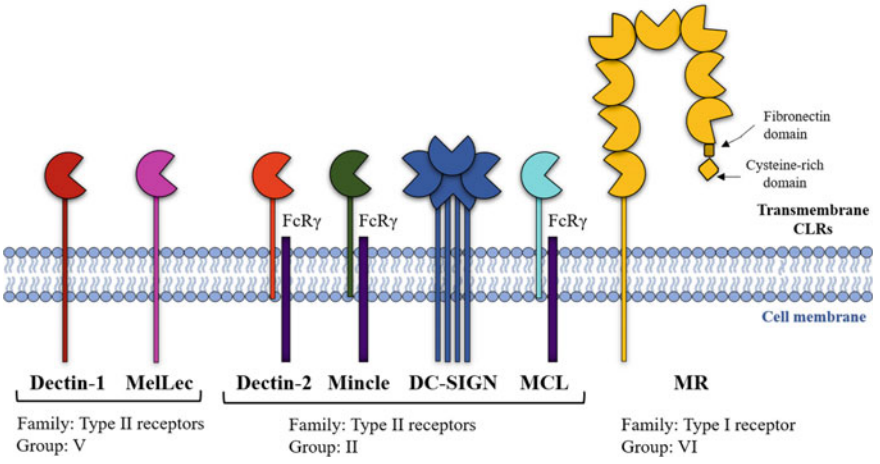

Fig. 1.3 Schematic representation of C-type lectin receptors. The structure of the CLRs described in the main text and the groups they belong to are depicted here

multiple CTLDs, and group II and V CLRs are type II transmembrane receptors with single extracellular CTLDs (Fig. 1.3). Some of these transmembrane receptors can trigger intracellular signalling directly or through association with other signalling molecules (for e.g. FcRγ) (Fig. 1.4), forming signalling heterodimeric and sometimes heterotrimeric complexes. An example of heterotrimeric complex is the recently described one formed between macrophage-inducible C-type lectin (Mincle)/macrophage C-type lectin (MCL)/FcεRI-γ (Lobato-Pascual et al. 2013). According to the signalling pathways they utilise, CLRs can be further divided into activation or inhibitory receptors.

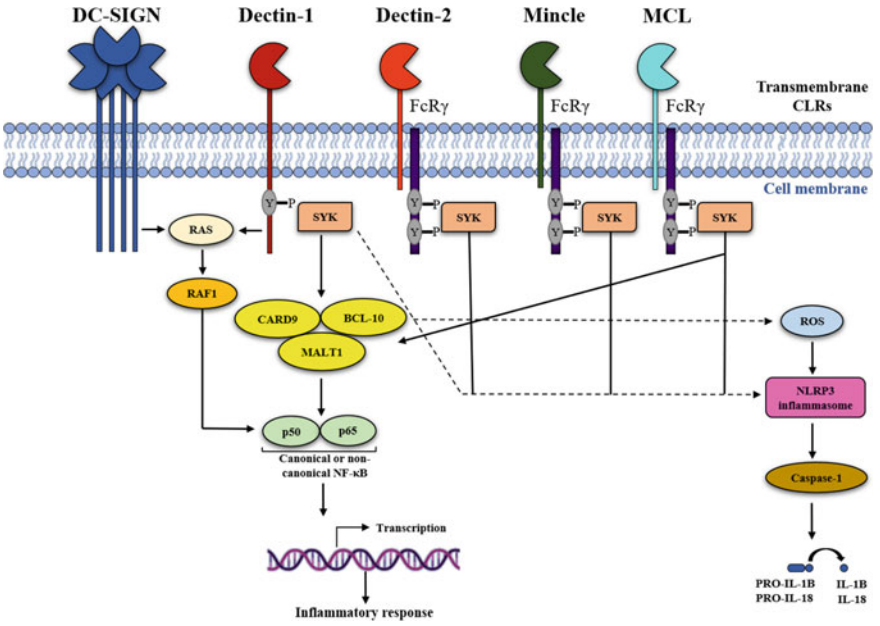

Fig. 1.4 Signalling pathways in innate recognition of fungi. The signalling pathways through which CLRs act are depicted here. Upon binding of activation CLRs (DC-SIGN, Dectin-1, Dectin-2, Mincle and MCL) to fungal PAMPs, they trigger intracellular signalling through an immunoreceptor tyrosine-based activation motif (ITAM) domain(s) located inside their cytoplasmic tails or via association with ITAM-bearing Fc receptor γ-chain (FcRγ) adaptor molecules. Phosphorylated ITAMs promote the recruitment of spleen tyrosine kinase (Syk) kinase, which is then phosphorylated resulting in the binding of caspase recruitment domain-containing protein 9 (CARD9), B-cell lymphoma/leukaemia 10 (BCL-10) and mucosa-associated lymphoid tissue 1 (MALT1). The resulting complex stimulates the canonical (c-Rel and p65) or the NF-κB-inducing kinase (NIK)-dependent non-canonical (RelB) NF-κB subunit and the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome. This leads to the production of reactive oxygen species (ROS) and the proteolytic activation of the pro-inflammatory cytokines IL-1β and IL-18 by caspase 1. Ligand recognition can also drive, in a cell and receptor specific manner, phagocytosis and the respiratory burst, which do not require transcription and in some cases are Syk-independent. Dectin-1 and DC-SIGN can also recruit the GTPase Ras proteins, which then activate the serine/threonine-protein kinase RAF1. RAF1 mediates the phosphorylation of the p65 subunit in a Syk-independent manner

Activation CLRs trigger intracellular signalling through immunoreceptor tyrosine-based activation motifs (ITAM; consensus sequence YxxL/I) located inside their cytoplasmic tails or via association with ITAM-bearing FcRγ adaptor molecules. Phosphorylated ITAMs promote the recruitment of spleen tyrosine (Syk) kinase (Gringhuis et al. 2009; Kerrigan and Brown 2011), resulting in a phosphorylation cascade leading to the activation of caspase recruitment domain-containing protein 9 (CARD9), B-cell lymphoma/leukaemia 10 (BCL-10) and mucosa-associated lymphoid tissue 1 (MALT1). The resulting complex stimulates transcription factors, including NF-κB, and results in chemokine and cytokine production. Unexpectedly, the phosphatase SHP-2 (previously associated with inhibitory receptors) has been implicated in stabilising Syk signalling necessary to control anti-*C. albicans* immunity (Deng et al. 2015). Activation of NF-κB can also be mediated by other signalling pathways that are Syk-independent. For example, activation of some CLRs, including Dectin-1 and dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), can recruit the GTPase Ras proteins, which then activate the serine/threonine-protein kinase RAF1. RAF1 mediates the phosphorylation of the p65 subunit of the NF-κB in a Syk-independent manner (Geijtenbeek and Gringhuis 2009). Ligand recognition can also drive, in a cell type and receptor-specific manner, phagocytosis, the respiratory burst and other cellular responses that do not require transcription and in some cases are Syk-independent (Drummond et al. 2011; Strasser et al. 2012) (Fig. 1.4).

Inhibitory CLRs signal via an immunoreceptor tyrosine-based inhibition motif (ITIM; consensus sequence I/V/L/SxYxxI/L/V) within their cytoplasmic tails. When these receptors are activated by their ligands, tyrosine and inositol phosphatases including SHP-1, SHP-2 and SHIP, are recruited that inhibit activation pathways, leading to suppression of cellular responses. Some CLRs like MelLec lack ITAM or ITIM or other domains with known function and their mechanisms of intracellular signalling are unknown. This was also thought to be true for the mannose receptor (MR) but its association with FcRγ chain was recently described (Rajaram et al. 2017).

1.4.1 CLRs in Antifungal Immunity

The essential nature of CLRs in antifungal immunity is highlighted by the fact that humans (and mice) lacking functional CARD9, the signalling molecule through which most of the CLRs signal (Fig. 1.4), are extremely susceptible to fungal infections (Drummond et al. 2011). Moreover, as we will discuss below, polymorphisms in CLRs are also associated with alterations in human susceptibility to fungal infection. The major CLRs that play a role in fungal recognition and induction of protective immunity are Dectin-1 (CLEC7A), Dectin-2 (CLEC6A), MR (CD206), Mincle

(CLEC4E), DC-SIGN (CD209), MCL/Dectin-3 (CLEC4D) and the recently discovered MelLec (CLEC1A). We will now provide a brief introductory description of each receptor.

Dectin-1

Dectin-1 belongs to the group V CLRs (Fig. 1.3) and is expressed as several isoforms generated by alternative splicing, but primarily as the full-length receptor and a receptor lacking the stalk region (Brown 2006; Heinsbroek et al. 2006). Dectin-1 is predominantly expressed on myeloid cells, including DCs, macrophages and neutrophils and on specific lymphocyte subsets and on B-cells in humans (Brown 2006). Dectin-1 recognises β-glucans of fungal cell walls in a Ca^{2+}-independent manner (Brown 2006) but also ligands on mycobacteria (Wagener et al. 2018), *Leishmania* (Lima-Junior et al. 2017) and invertebrate tropomyosin (Gour et al. 2018). In addition, this receptor recognises several endogenous ligands, including vimentin and galectin-9 (Thiagarajan et al. 2013; Daley et al. 2017). Dectin-1 utilises an ITAM-like motif in its cytoplasmic tail to trigger intracellular signalling through the Syk/CARD9 pathway, but is also able to trigger several other pathways, including RAF1 (Fig. 1.4). Dectin-1 signalling requires clustering into a phagocytic synapse, where regulatory tyrosine phosphatases CD45 and CD148 are excluded. Several other regulators have also been described, including SHIP-1 (Blanco-Menéndez et al. 2015), and internalisation of ligand baring particles is thought to cause cessation of signalling (Hernanz-Falcón et al. 2009).

Dectin-1 is involved in inducing or regulating several cellular and immunological responses. For example, Dectin-1 can induce phagocytosis, the respiratory burst leading to production of reactive oxygen species (ROS) and regulate NETosis. It can also induce the production of cytokines and chemokines, and other inflammatory mediators. Finally, it can regulate the development of Th1 and Th17 adaptive immune responses (Goodridge et al. 2009; Kerrigan and Brown 2009).

MelLec

MelLec has structure similar to that of Dectin-1 and is part of the group V CLRs (Fig. 1.3). Unlike other fungal sensing CLRs, murine MelLec is not expressed on myeloid cells ($CD45^-$) but it is expressed on endothelial ($CD31^+$) cells. Interestingly, MelLec is also expressed on a novel subpopulation of $CD31^+$ cells that co-express the epithelial marker ($EpCAM^+$); these double-positive cells ($CD31^+EpCAM^+$) were only found in the mouse lung and liver (Stappers et al. 2018). In humans, this receptor is expressed on endothelial and myeloid cells, including DCs (Colonna et al. 2000; Sobanov et al. 2001; Sattler et al. 2012). MelLec recognises the naphthalene-diol unit of 1,8-DHN melanin found in several fungal species, including *A. fumigatus* and *Fonsecaea pedrosoi*. This recognition is specific, as MelLec does not recognise other melanin biosynthetic pathways, such as L-DOPA melanin synthesised in *Candida* or *Cryptococcus.* Despite its structural similarity with Dectin-1, MelLec lacks conserved motifs in its cytoplasmic tail and its mechanisms of intracellular signalling are not yet known.

Dectin-2

Dectin-2 is a member of the group II of CLRs (Fig. 1.3), and is expressed on tissue macrophages, DCs, neutrophils, Langerhans cells (LCs) and Kupffer cells (Shiokawa et al. 2017). Dectin-2 recognises mannose-based structures in a Ca^{2+}-dependent manner but has greatest affinity for high-mannose structures (e.g. $Man_9GlcNAc_2$) (McGreal et al. 2006). Dectin-2 forms a heterodimer with MCL (see later), which allows optimal ligand recognition (Zhu et al. 2013). Dectin-2 recognises a wide variety of ligands including numerous fungal species, as well as bacteria (e.g. *Mtb*), schistosome egg antigen and house dust mite allergens (HDM) (Ritter et al. 2010; Parsons et al. 2014; Shiokawa et al. 2017). Dectin-2 lacks a cytoplasmic tail with signalling domains, but like other receptors in this group, dimerizes with the ITAM-containing FcRγ chain for surface expression and intracellular signalling (Sato et al. 2006).

Cellular and immunological functions of Dectin-2 include phagocytosis, production of cytokines and ROS. Dectin-2 has also been shown to mediate extracellular trap formation in plasmacytoid DCs (pDCs) in response to *Aspergillus* hyphae (Loures et al. 2015). Dectin-2 has been implicated in allergic airway inflammation, through stimulation of cysteinyl leukotrienes (Barrett et al. 2009). Indeed, mice lacking Dectin-2 have decreased levels of HDM-mediated Th17 and Th2 cell differentiation as well as reduced inflammation of the airways (Plantinga et al. 2013; Norimoto et al. 2014). This receptor is also involved in mounting protective Th17 and Th1 responses to fungi (Wang et al. 2017).

Mincle

Mincle belongs to the Group II CLRs and is present on macrophages, DCs and neutrophils (Williams 2017) (Fig. 1.3). The CRD binds endogenous ligands (SAP130, sterols and β-glucosylceramides), mycobacterial ligands (such as cord factor, thermoduric bacteria) and fungal ligands (e.g. mannosyloxystearyl mannitol, galectin and β-gentiobiosyl diglycerides). Fungal pathogens recognised by Mincle include *C. albicans*, *P. jirovecii*, *Fonsecaea* and *Malassezia*. Mincle expression is enhanced following stimulation by fungal and microbial components through TLRs signalling (Patin et al. 2017). Mincle forms a heterotrimeric complex with MCL and FcRγ, which is required for surface expression, enhances phagocytosis, and stimulates signalling via the NF-κB pathway (Lobato-Pascual et al. 2013) (Fig. 1.4).

DC-SIGN

DC-SIGN, another member of group II CLRs (Fig. 1.3), has eight orthologues in mice mDC-SIGN, L-SIGN, DC-SIGNR1,2-4 and DC-SIGNR6-8 which differ in structure and expression patterns (Park et al. 2001; Koppel et al. 2005; Powlesland et al. 2006). Humans have two orthologues of this receptor: DC-SIGN and liver/lymph node-specific ICAM-3-grabbing integrin (L-SIGN) (Soilleux et al. 2000; Bashirova et al. 2001). DC-SIGN is mainly present on the surface of immature DCs in peripheral tissue, mature DCs in lymphoid tissue, and some subsets of macrophages (Koppel et al. 2005; Krutzik et al. 2005). DC-SIGN recognises mannose structures, such as galactomannans, in a Ca^{2+}-dependent fashion and recognises a range of endogenous

ligands and pathogens, including viruses (e.g. HIV, Hepatitis C, Ebola), bacteria (*Mtb*), *Leishmania* and fungi (e.g. *C. albicans*, *Chrysosporium tropicum*, *A. fumigatus*) (Garcia-Vallejo and van Kooyk 2013). DC-SIGN recognises allergens from peanut, HDM (e.g. Derp1), dog and the Bermuda grass pollen allergen (Salazar et al. 2013). DC-SIGN possesses several internalisation motifs and signals via RAF1 (Gringhuis et al. 2007) (Fig. 1.4).

Most studies on DC-SIGN have been performed using human cells in vitro. Through these types of analyses, DC-SIGN has been associated with allergy as DC-SIGN-deficient DCs biased towards a Th2 cell polarisation in autologous DC-T cell cocultures (Emara et al. 2012). In addition, DC-SIGN can be cleaved by the protease allergen Derp1, which could contribute to driving allergic responses (Furmonaviciene et al. 2007). The cellular and immunological responses of DC-SIGN orthologues in mouse models are still to be examined.

Mannose Receptor (MR)

The MR is part of the group VI CLRs (Fig. 1.3) and can be proteolytically cleaved to form a soluble version. Interestingly the cleavage of the MR can be induced following Dectin-1 signalling (Gazi et al. 2011). This receptor is primarily expressed on tissue macrophages and some DC subtypes. The CTLDs bind to branched N-linked mannans in a Ca^{2+}-dependent manner, while the CR and FN-II domains have both exogenous and endogenous ligands (sulphated carbohydrates found in lymphoid and kidney tissues and collagen I–IV, respectively) (Martinez-Pomares 2012). The MR has also been reported to bind to α-1,3-glucan and chitin (Cambi et al. 2008; Romani 2011), and *P. jirovecii* glycoprotein A (O'Riordan et al. 1995). Other exogenous ligands of the MR include viruses (HIV, Dengue virus, HBV), bacteria (*Mtb*, *Klebsiella*, *S. pneumonia*), allergens (e.g. HDM), parasites (helminths, *Trichuris muris*) and fungi (e.g. *C. neoformans*, *P. jirovecii*) (Martinez-Pomares 2012). The short intracellular cytoplasmic tail of the MR has no known signalling motifs, although it was recently shown to associate with the FcRγ receptor (Rajaram et al. 2017).

The MR has been implicated in endocytosis, phagosomal trafficking, cross presentation, and promotion of antifungal Th17 responses (van de Veerdonk et al. 2009; Romani 2011). This receptor is also involved in allergy and asthma as it is required for allergen-induced Th2 cell polarisation, it is highly expressed in the DCs of allergic patients (Deslée et al. 2002; Royer et al. 2010), and the MR C-type 1 (*MRC1*) gene has been identified as a candidate for allergen (Li et al. 2007; Hattori et al. 2009).

1.4.2 CLRs in Immunity to Candida albicans

C. albicans is normally found in the microflora of 30–70% of healthy individuals, without causing any disease, including the oral cavity, vagina, intestinal mucosa and skin (Rizzetto et al. 2015). However, *C. albicans* is also an opportunistic human fungal pathogen when the immune system of the host is compromised. This fungus can

exist as yeast, hyphae and pseudohyphae (filamentous forms), and morphogenetic switching is thought to be a virulence factor (Saville et al. 2003; Wächtler et al. 2011). *C. albicans* can infect oral and vaginal mucosae, skin and nail beds causing chronic mucocutaneous candidiasis (CMC), which arises due to dysfunction of T cells. *C. albicans* can also cause invasive candidiasis upon rupture of the host's physical barriers (e.g. skin and gut mucosal barriers) and/or modulation of host immunity. This pathogen is primarily thought to cross the GI tract allowing dissemination of the fungus into the bloodstream and deep tissues (Gow et al. 2012). Following *Staphylococcus epidermidis* and *Staphylococcus aureus*, invasive candidiasis is the fourth most common cause of bloodstream hospital infections. The mortality rate of invasive candidiasis is 30–40% (Perlroth et al. 2007).

1.4.2.1 *Candida* Infections and Dectin-2

Dectin-2 has been implicated in anti-*Candida* immunity. It recognises yeast and hyphae of *C. albicans* and plays an important role in driving the stimulation of cytokines upon *C. albicans* infection (Sato et al. 2006; Bi et al. 2010; Saijo et al. 2010). In response to infection, Dectin-2 forms heterodimers with MCL, and this heterodimeric complex binds to α-mannans more efficiently, leading to the induction of pro-inflammatory responses (Zhu et al. 2013). Dectin-2 has been found to contribute slightly to the phagocytosis of *C. albicans* (Bi et al. 2010) and neutrophils lacking Dectin-2 produced less ROS, which is critical for the killing of *Candida* (Ifrim et al. 2014).

1.4.2.2 *Candida* Infections and MCL

MCL was found to be a critical CLR for recognising and protecting against *C. albicans* as $MCL^{-/-}$ mice were susceptible to systemic candidiasis (Zhu et al. 2013). Nothing more is known regarding the role of MCL during *Candida* infections in humans.

1.4.2.3 *Candida* Infections and MR

C. albicans is also sensed by the mannose receptor. Despite the lack of phenotype in MR KO mice (Lee et al. 2003), the MR was initially recognised as an opsonin-independent phagocytic receptor able to bind and phagocytose un-opsonised *C. albicans* yeast particles (Porcaro et al. 2003) and zymosan particles (Harris et al. 1992). In contrast, other studies have suggested that the MR is implicated in live *C. albicans* uptake, it is recruited during phagosome maturation and is involved in cytokine release, e.g. monocyte chemoattractant protein 1 (MCP-1), TNF and IL-17 (Netea

et al. 2006; Heinsbroek et al. 2008). No MR SNPs associated with increased susceptibility to *Candida* infections have been identified in humans. Thus, more studies are required to decipher the role of MR in protection against *C. albicans.*

1.4.2.4 *Candida* Infections and Dectin-1

The most well studied CLR against *C. albicans* infections is Dectin-1. It is involved in recognition and uptake of *C. albicans*, and it is able to stimulate ROS and nitric oxide (NO) production, phagocytosis and cytokine release (e.g. TNF, IL-6) in response to *C. albicans* infection in vitro (Drummond and Brown 2011; Pinke et al. 2016).

Dectin-1 has been found to be critical for protecting against systemic *C. albicans* infections in the GI tract. This receptor is crucial for activating $CD4^+$-T cell, but not $CD8^+$-T cell, responses in these tissues (Drummond et al. 2016). Dectin-1 is also required for the killing of *C. albicans* and the proper production of cytokines upon infection in the GI tract (Galès et al. 2010; Vautier et al. 2012). Dectin-1 also plays a critical role in protecting against candidiasis during inflammatory bowel disease (IBD) and colitis (Iliev et al. 2012). In humans, a single nucleotide polymorphism in CLEC7A (rs2078178) is significantly associated with ulcerative colitis (Iliev et al. 2012).

However, the role of Dectin-1 in protection against *Candida* infections in vivo depends on the site of infection, and strain of mouse and fungus. Dectin-1 has been reported to be essential for controlling vaginal and gastrointestinal candidiasis in C57BL/6 but not BALB/c mice, phenotypes that may be related to the isoforms of Dectin-1 (full-length Dectin-1A or the stalkless Dectin-1B) predominant in these mice (Heinsbroek et al. 2006; Carvalho et al. 2012). There are also conflicting reports describing the importance of Dectin-1 during systemic infection, however this has been linked to differential adaptations of different fungal strains (Saijo et al. 2007; Taylor et al. 2007; Netea et al. 2010; Marakalala et al. 2013).

Dectin-1 also appears to be associated with protection against chronic mucocutaneous candidiasis (CMC), which is caused by defective production of IL-17 and IL-22 as well as impaired Th17 cell differentiation. Using a skin mouse infection model, it was discovered that different morphological forms of *C. albicans* facilitate the development of different T helper responses that are protective for either systemic or cutaneous infection in a Dectin-1-dependent or independent manner. Specifically, *C. albicans* yeasts mediated a protective Th17 response against cutaneous infection via Dectin-1 (Kashem et al. 2015). Dectin-1 is crucial for defence against mucosal *C. albicans* infection in humans. The Tyr238X Dectin-1 polymorphism (completely lacking Dectin-1 surface expression) has been associated with reduced Th17 responses and increased susceptibility to CMC (Ferwerda et al. 2009). Indeed, stem cell transplant patients carrying this polymorphism had abrogated IL-1β production and increased colonisation with *Candida* (Plantinga et al. 2009).

Dectin-1 has been implicated in the development of liver cirrhosis. Chronic exposure of ethanol in mice led to fungal dysbiosis in the intestine and increased the translocation of β-glucans into the systemic circulation (Yang et al. 2017). This

β-glucan induced inflammatory responses in Kupffer cells occurs in a Dectin-1-dependent manner. Associated phenotypes were also observed in human alcoholic patients with cirrhosis (Yang et al. 2017).

C. albicans can enter the skeletal tissue, causing osteomyelitis. This infection is hard to treat and causes significant morbidity (Gamaletsou et al. 2012). It was recently reported that Dectin-1 might contribute to the resolution of *C. albicans* osteomyelitis by inducing Nav1.8-positive-nociceptors, which prevent osteomyelitis and osteoporosis in response to β-glucan (Maruyama et al. 2017).

Other CLRs that we have not described here that have been implicated in host defence against *C. albicans* infections include dendritic cell natural killer lectin group receptor-1 (DNGR-1; CLEC9A). DNGR-1 was recently found to be required for controlling systemic *C. albicans* infection in mice and it reduced tissue damage by controlling the recruitment of neutrophils to *C. albicans* infected tissues (Cano et al. 2018).

1.4.3 CLRs in Immunity to Aspergillus fumigatus

The saprophytic fungus *A. fumigatus* belongs to the genus *Aspergillus* that is comprised of about 200 species (Gugnani 2003). *Aspergillus* species are found in decaying vegetation and soil, and contribute to a great extent to the recycling of carbon and nitrogen (Van De Veerdonk et al. 2017). Only a few *Aspergillus* species cause infections in humans, with *A. fumigatus* being the most common. *A. fumigatus* is very abundant in the environment, releasing spores (conidia) which are inhaled. It is an opportunistic pathogen that causes disease in people whose lung function or immune defences are impaired. About 1–15% of cystic fibrosis (CF) patients and 2% of asthma patients, develop allergic bronchopulmonary aspergillosis (ABPA), a pulmonary disease caused by hypersensitivity of these patients to antigens of *Aspergillus* (especially *A. fumigatus*). It may also contribute to the pathogenesis of CF (Escobar et al. 2016). In immunocompromised individuals, *A. fumigatus* hyphae can penetrate pulmonary tissues causing invasive aspergillosis (IA), which is the most severe disease caused by *Aspergillus* spp. (https://www.gaffi.org/why/fungal-disease-frequency/). Immunocompromised people affected by IA include, hematopoietic stem cell transplant (HSCT) patients, solid-organ (particularly lung) transplantation and people with genetic immunodeficiencies, e.g. chronic granulomatous disease (CGD) (Denning 1998). Emerging populations that are high in risk for IA are patients admitted to the intensive care unit (ICU) (up to 7% incidence) and chronic obstructive pulmonary disease (COPD) patients (Kousha et al. 2011; Schlamm et al. 2013). Despite the availability of antifungal drugs, mortality rates of IA exceed 50% in neutropenic patients and reaches 90% in HSCT recipients (Kousha et al. 2011).

1.4.3.1 *Aspergillus* Infections and Dectin-1

Dectin-1 was found to play an important role on human immature dendritic cells (iDCs) and myeloid DCs (mDCs) during *A. fumigatus* infection, as inhibition of this receptor resulted in decreased production of pro-inflammatory cytokines (Mezger et al. 2008; Hefter et al. 2017). However, the role of Dectin-1 in human pDCs during *A. fumigatus* infection is contradictory; this CLR has been shown to facilitate the uptake and clearance of *A. fumigatus* spores by human pDCs (Maldonado et al. 2015), but it has also been demonstrated that human pDCs recognise *A. fumigatus* hyphae via Dectin-2 but not Dectin-1 (Loures et al. 2015). The different results obtained from the two studies could be attributed to different forms of *A. fumigatus* and/or different strains used.

The requirement of Dectin-1 in protection against IA has been demonstrated by murine studies. For example, Dectin-$1^{-/-}$ mice were susceptible to *A. fumigatus* infection as they had increased fungal burdens in their lung and most of them died within 5 days of infection. There was also a defective recruitment of neutrophils, uncontrolled growth of *A. fumigatus* and reduced release of cytokines (e.g. IL-17) and chemokines in the lungs of Dectin-$1^{-/-}$ mice (Werner et al. 2009). Polymorphisms of Dectin-1, including the Dectin-1 TyrY238X polymorphism, have been linked to susceptibility to IA in transplant patients (Fisher et al. 2017). Indeed, polymorphonuclear cells (PMCs) from individuals carrying the polymorphism released less cytokines such as interferon-γ (IFNγ) (Cunha et al. 2010; Chai et al. 2011).

As mentioned earlier, exposure to *A. fumigatus* increases the severity of asthma in patients. Although Dectin-1 has been explored in asthma, its role is still unclear. Dectin-1 has been found to provide lung protection in mice via IL-22 (a cytokine highly increased in asthmatic patients) against *A. fumigatus* at early innate responses (Gessner et al. 2012). However, following chronic fungal exposure, this CLR might also enhance lung inflammation via release of this cytokine (Lilly et al. 2012). Further investigation is thus needed to identify the role of Dectin-1 in asthma.

Aspergillus species can also cause a disease of the cornea known as fungal keratitis, which can lead to severe visual defects and blindness in immunocompromised individuals. Dectin-1 has been found to play a role in this disease; it is involved in regulating neutrophil recruitment, release of IL-1β and other cytokines (e.g. CXCL1), and *A. fumigatus* killing (Leal et al. 2010; Xu et al. 2015; Yuan et al. 2017).

1.4.3.2 *Aspergillus* Infections and Dectin-2

Recently, Dectin-2, Dectin-1 (in an isoform specific manner) and Mincle were found to be important in the clearance of *A. fumigatus* from the lung of CF patients; cleavage of these three CLRs by neutrophil elastase (NE) (which is upregulated in the lungs of CF patients) and/or *A. fumigatus*-derived proteases, led to impaired antifungal protection, which might be also contributing to disease development in these patients (Griffiths et al. 2018). In a model of *A. fumigatus* keratitis, Dectin-2 enhanced the expression of IL-17RC on murine and human neutrophils (Taylor et al. 2014). Lastly,

Dectin-2 is also expressed in the lungs of patients with IA or ABPA, where it might be collaborating with the MR (Sun et al. 2013).

1.4.3.3 *Aspergillus* Infections and MR

The mannose receptor plays a role in protection against *A. fumigatus* in human corneal epithelial cells (HCECs) as upon stimulation of these cells with *A. fumigatus*, the mRNA and protein expression levels of the MR were upregulated, and this CLR was found to be required for inducing the production of inflammatory cytokines by these cells (Wang et al. 2016). No human polymorphisms in MR have been associated with infection, nor are there published studies examining the impact of MR-deficiency in mouse models.

1.4.3.4 *Aspergillus* Infections and DC-SIGN

A. fumigatus is also recognised by DC-SIGN, and although limited studies have been carried out, this receptor is able to directly mediate the phagocytosis of conidia in IL-4 treated macrophages and monocyte derived (MD)-DCs (Serrano-Gomez et al. 2004). Similarly, DCs overexpressing DC-SIGN phagocytosed *A. fumigatus* more effectively and produced higher levels of Th1 cytokines (Li et al. 2018). Interestingly, this receptor appears to recognise live but not heat-killed *A. fumigatus* conidia (Serrano-Gómez et al. 2005). Some DC-SIGN (rs4804800, rs11465384, rs7248637 and rs7252229) polymorphisms are associated with an elevated risk of developing IA, but this requires validation (Sainz et al. 2012). No studies in mouse models have been conducted so far.

1.4.3.5 *Aspergillus* Infections and MelLec

The novel CLR, MelLec, recognises melanized *A. fumigatus* conidia and provides protection against disseminated *A. fumigatus* infection. Mice lacking this receptor had impaired cytokine production (IL-1β, CCL2 and KC) and elevated fungal loads and were more susceptible to infection. A missense single nucleotide polymorphism (Gly26Ala) in the cytoplasmic tail of human MelLec is associated with reduced IL-8 and IL-1β cytokine release from macrophages, and stem cell transplant patients carrying this polymorphism were more susceptible to disseminated *A. fumigatus* infection (Stappers et al. 2018).

1.4.4 CLRs in Immunity to Cryptococcus neoformans

C. neoformans is an encapsulated, basidiomycete fungus that is typically found in trees, soil and pigeon droppings. It is a human pathogen responsible for causing cryptococcosis and cryptococcal meningitis. This fungus has three serotypes (A, D and AD) and two varieties (*C. neoformans* variety *grubii* (serotype A) and *C. neoformans* variety *neoformans* (serotype D)), which are found in different geographic regions and have different virulence factors to infect humans (Hagen et al. 2015). *C. neoformans* can form titan cells during infection and due to their gigantic size, their clearance by phagocytes is impossible. These cells help the fungus to survive and spread throughout the central nervous system (CNS), and they also exhibit increased resistance to drugs (Zaragoza et al. 2010). Cryptococcosis occurs after inhalation of desiccated yeast cells or spores into the lungs, where they proliferate and disseminate to the CNS, causing meningoencephalitis (Buchanan and Murphy 1998). Spores can also infect the kidneys and liver (Perfect 2005). Cryptococcosis mainly affects immunocompromised persons, such as HIV infected patients (Idnurm et al. 2005; Hagen et al. 2015). Globally about 220,000 million cryptococcosis cases occur among HIV patients worldwide each year, with the majority occurring in sub-Saharan Africa, leading to 181,000 deaths (https://www.cdc.gov/fungal/diseases/cryptococcosis-neoformans/statistics.html). Failure to treat disease of the CNS is fatal in 100% of cases (Buchanan and Murphy 1998).

Very little is known regarding the roles of CLRs in protection against this fungus, and sometimes the results of different studies are contradictory. Thus, more research is required to understand how *Cryptococcus* is recognised by our innate immune system.

1.4.4.1 *Cryptococcus* Infections and Dectin-1

The role of Dectin-1 in protection against cryptococcosis has been debated. This CLR was found to be redundant for providing protection against *C. neoformans*, as mice lacking Dectin-1 were not susceptible to *C. neoformans* infection (Nakamura et al. 2007). Spores of *C. neoformans* expose β-glucan and have been found to be taken up by alveolar macrophages via an interaction with Dectin-1 (Giles et al. 2009). However, it was later reported that Dectin-1 is not necessary for the phagocytosis of *C. neoformans* spores (Walsh et al. 2017). Finally, a recent study showed that Dectin-1 together with Dectin-2 facilitates the uptake of *C. neoformans* (Lim et al. 2018).

1.4.4.2 *Cryptococcus* Infections and Dectin-2

In vitro experiments have indicated a role for Dectin-2 in inflammatory responses to *Cryptococcus*, as cells lacking Dectin-2 released significantly less TNF and IL-12. However, in mice, Dectin-2 appears to play a minor role during infection, as inflammatory responses and susceptibility to infection were unaltered, although there were slight alterations in terms of increased Th2 responses and increased production of mucin (glycoprotein component of the mucous) (Nakamura et al. 2015).

1.4.4.3 *Cryptococcus* Infections and MR

The mannose receptor is required for host defence in cryptococcosis; this CLR is necessary for binding and phagocytosis of *C. neoformans* by DCs (Syme et al. 2002) but it is redundant for the uptake of mannoproteins upon infection (Dan et al. 2008). $MR^{-/-}$ mice exhibited increased susceptibility to pulmonary *C. neoformans* infection and had high fungal burdens in their lung. Furthermore, during infection, the MR is required for the development of mannoprotein-dependent $CD4^{+}$-T cell responses (Dan et al. 2008).

1.4.4.4 *Cryptococcus* Infections and MCL

Contradictory results have been obtained regarding the role of MCL during *C. neoformans* infections. MCL recognises glucuronoxylomannan (GXM), which is the main component of *Cryptococcus* capsule, and mediates the release of pro-inflammatory cytokines by *C. neoformans* infected bone marrow derived macrophages (BMDMs) (Huang et al. 2018). However, $MCL^{-/-}$ macrophages and DCs were able to phagocytose *C. neoformans* in vitro. In addition, no differences were observed in survival rate, cytokine release and leukocyte recruitment between wild-type and $MCL^{-/-}$ mice in response to pulmonary infection with serotype A or serotype D strains of *C. neoformans*, suggesting that MCL is not required for host protection against cryptococcosis (Campuzano et al. 2017). In contrast, a recent report describes increased susceptibility in $MCL^{-/-}$ mice upon infection with serotype AD (hybrid) of *C. neoformans* (Huang et al. 2018). Thus, the impact of MCL deficiency may differ depending on the serotype of *C. neoformans.*

1.4.5 CLRs in Immunity to Pneumocystis jirovecii

P. jirovecii is an opportunistic ascomycetous fungus that causes life-threatening pneumonia. This fungus is not a human commensal. As it can only grow inside the host lung, culture conditions have not been found so far, making it difficult/impossible to grow *P. jirovecii* in vitro (Thomas and Limper 2004). About 20% of healthy

individuals get this fungus, which does not cause any disease and it is effectively controlled by the immune system (Medrano et al. 2005). *P. jirovecii* causes disease in immunocompromised patients, especially those with low count of $CD4^+$-T cells (Phair et al. 1990); thus, 40% of immunocompromised people who develop *Pneumocystis* pneumonia are HIV patients. The mortality rate of *Pneumocystis* pneumonia ranges between 30–60% in immunocompromised patients, and 10–20% among HIV patients (Thomas and Limper 2004).

Very little information is available regarding the role of CLRs in *Pneumocystis* infection, e.g. nothing is known about the role of MCL, and some of the CLRs known to recognise this fungus are redundant; more research is necessary to fully understand the innate recognition of this fungus.

1.4.5.1 *Pneumocystis* Infections and MR

One of the first CLRs described to recognise *P. jirovecii* was the MR (Ezekowitz et al. 1991) as it bound to gpA (O'Riordan et al. 1995), and this CLR was shown to be sufficient to induce the phagocytosis of *P. jirovecii* (Ezekowitz et al. 1991). Also, alveolar macrophages (AMs) (first immune cells responsible for the killing of *P. jirovecii* in the lung) from HIV patients were less efficient in binding and killing *P. murina* (a mice-associated species) as the MR was downregulated on these cells (Koziel et al. 1998). A soluble MR immuno-adhesin (sMR-Fc) has been generated which binds to *P. jirovecii*, it participates in phagocytosis via its Fc-portion and augments the phagocytosis of *P. jirovecii* by human polymorphonuclear cells (Stehle et al. 2000). However, *Pneumocystis* promotes the cleavage of the MR from AMs, releasing the sMR; because sMR binds to the organisms in the alveoli, the non-opsonic phagocytosis of this receptor is impeded (Fraser et al. 2000). Notably, one of the mechanisms that *Pneumocystis* has developed to evade immune killing is the release of its gpA, which competes for the MR on the surface of AMs preventing phagocytosis (Lasbury et al. 2004). In vivo studies demonstrated that more phagocytes were recruited into the alveoli of $MR^{-/-}$ mice during infection with *P. murina* but did not develop any signs of disease (Swain et al. 2003).

1.4.5.2 *Pneumocystis* Infections and Dectin-1

Dectin-1 contributes to protection against *P. jirovecii*. In vitro experiments have shown that cysts of *P. jirovecii* are recognised by Dectin-1, facilitating uptake and clearance by AMs. Indeed, overexpression of Dectin-1 in macrophages enhanced binding to this fungus (Steele et al. 2003). In vivo, Dectin-$1^{-/-}$ mice exhibited high susceptibility and displayed impaired generation of ROS in response to *Pneumocystis* lung infection. However, no differences were observed in cytokine release between Dectin-$1^{-/-}$ and wild-type mice (Saijo et al. 2007).

1.4.5.3 *Pneumocystis* Infections and Mincle

Mincle was recently found to be able to recognise *P. murina* (Kottom et al. 2017). Overexpression of Mincle in mouse macrophages enhanced their binding to this fungus and increased the phosphorylation of Syk kinase. Mincle$^{-/-}$ mice had impaired cytokine production in their lungs and decreased killing ability. However, the susceptibility of these mice was not altered during infection. Mincle$^{-/-}$ mice lacking CD4 succumbed to *P. murina* infection faster than wild-type mice lacking CD4. Thus, Mincle is required for the induction of host responses against *Pneumocystis* in immunosuppressed mice. Notably, Mincle may regulate expression of other CLRs including Dectin-1, Dectin-2 and MCL during *Pneumocystis* infection (Kottom et al. 2017).

1.4.5.4 *Pneumocystis* Infections and Dectin-2

Dectin-2 might also be required for host defence against *P. jirovecii* as AMs from mice lacking this receptor could not trigger Syk kinase and they produced significantly lower levels of TNF and IL-6. However, Dectin-2 may not be required for protection against *Pneumocystis* in immunosuppressed mice as Dectin-2$^{-/-}$ mice lacking CD4 were not susceptible to *Pneumocystis* infection (Kottom et al. 2018).

1.5 Conclusion and Future Directions

CLRs are critical for the protection against fungal infection. Although we have learned a great deal about the roles and functions of CLRs in antifungal immunity in general, there is much we still do not understand about their roles during infection with specific pathogens. Moreover, we are also just starting to understand the mechanisms that fungal pathogens have developed to escape recognition by these receptors. For example, fungi can alter their morphology and cell wall composition, as well as mask key components, such as β-glucan. A greater understanding of how CLRs mediate responses to fungal pathogens offers great promise for the development of new diagnostic tools, vaccines and immunotherapies, which are desperately needed to combat these devastating infections.

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