Recognition of non-self-polysaccharides by C-type lectin receptors dectin-1 and dectin-2

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Dendritic cells detect specific pathogens

Dendritic cells (DC) and macrophages are antigen-presenting cells (APC) that normally reside in peripheral tissues, such as skin and mucous membranes, where they sample soluble antigens and insoluble pathogens through endocytosis and phagocytosis. After internalization of these molecules, DC and macrophages activate a number of mechanisms that can process or kill pathogens intracellularly, including reactive oxygen species and nitric oxide (NO). This process constitutes part of the immune response termed “innate immunity,” an immediate defense system, that recognizes and promptly responds to pathogens in a largely generic manner. Activated APC also travel from peripheral tissues to draining lymph nodes after internalizing pathogens. During this migration, APC digest pathogen-derived antigens and display these antigens on their cell surface in association with major histocompatibility molecules. Within draining lymph nodes, antigen-specific T cells encounter APC and become activated, leading to the generation of helper and effector T cells. This “adaptive immunity” confers long-lasting protection to the host by producing antigen-specific antibodies (Th2-type immunity) and generating cytotoxic T cells (Th1-type immunity). Therefore, in great part, innate immunity conditions adaptive immunity.

Accumulating evidence indicates that the initial contact of DC with pathogens determines the type, balance, and strength of the subsequent adaptive response (Th1 versus Th2-type). A critical question in this process is whether DC are able to distinguish one pathogen from another, and thereby tailor immune responses to specifically target particular microbes. This question was partially addressed by a study that infected DC with three classes of pathogens: E. coli (representing bacteria), Candida albicans (fungi), and influenza virus. Changes in the gene expression patterns of infected DC were then analyzed by genome-wide microarray studies. Interestingly, DC displayed pathogen-specific programs of gene expression, although a shared core gene expression response was noted (Huang et al. 2001). Specific reprograming of gene expression conferred DC with the ability to induce pathogen-specific responses. Although the mechanism of pathogen recognition by DC remains poorly understood, somehow these cells are able to differentiate among microbes and elicit tailored responses.

Pattern recognition receptors

Studies over the last several decades have demonstrated that DC and macrophages recognize diverse pathogens by expressing a variety of receptors that interact with corresponding surface molecules on infectious agents. These receptors can be sorted into two major groups. The first group includes complement and Fc receptors that bind microbes coated with opsonin (host serum or tissue-fluid proteins including complement and immunoglobulin) (Gordon 2002). These receptors are unable to distinguish among varying pathogen types. By contrast, the second receptor group interacts directly with molecular patterns displayed by pathogens but not in host cells (Barton and Medzhitov 2002; Gordon 2002). These structures are referred to as “pathogen-associated molecular patterns (PAMPs)” (Janeway 1992). Host receptors for PAMPs are thus deemed, “pattern recognition receptors (PRR).” Recognition of PAMPs by PRRs enables cells of the innate immune system to not only perform microbial uptake and killing but also to modulate gene expression leading to an adaptive immune response targeted at the individual pathogen. In recent years, numerous PRRs have been identified and studied. These may recognize PAMPs composed of protein, lipid, and carbohydrate residues. Three groups of PRRs that have provided researchers with numerous insights into the development of innate immunity are the toll-like receptors (TLR), nucleotide oligomerization domain (NOD)-like receptors (NLR), and C-type lectin receptors (CLR).

TLRs consist of extracellular domains that mediate recognition of PAMPs that are usually composed of proteins. Upon ligation, TLRs ignite a signaling cascade mediated by intracellular adaptors that activate transcription factors such as NF-κB and interferon-regulatory factor 3 (IRF3), among others, and thereby direct TLR-specific patterns of gene expression and the production of cytokines and chemokines that prime innate and...
adaptive immunity (Underhill 2003; Netea et al. 2004; Akira et al. 2006; Willment and Brown 2007).

Nod-like receptors are similar to TLRs in utilizing a structurally related ligand-binding domain to recognize protein-based PAMPs and subsequently activating signaling pathways that mediate the production of inflammatory cytokines and chemokines. NLRs, however, are cytoplasmic receptors and bind both microorganisms after they invade the host cell cytoplasm and the pathogenic products released or transported into the cytoplasm during phagocytosis and degradation (Fritz et al. 2006; Strober et al. 2006; Kufer et al. 2007).

In contrast to TLRs and NLRs, CLRs recognize PAMPs composed of carbohydrate residues. Still, CLRs utilize many of the same signaling mechanisms as TLRs and NLRs in establishing the innate and adaptive immune defense. In fact, recent evidence suggests that PRRs of many types collaborate to recognize and combat pathogens (Gantner et al. 2003; Taylor et al. 2004; Netea et al. 2006; Trinchieri and Sher 2007; Underhill 2007). It may be that different PRRs work together not only to sense various individual microorganisms, but also to adjust immune defenses to the evolving virulence structures displayed by a single microorganism during the course of infection.

The purpose of this review is to outline the role of CLRs in innate immunity, focusing on dectin-1 and dectin-2, which we discovered and continue to characterize functionally.

C-type lectin receptors

C-type lectin receptors are a large superfamily of proteins that were defined originally by their ability to bind carbohydrates in a Ca\(^{++}\)-dependent manner (Weis et al. 1998). Soluble and membrane-bound receptors are included in this superfamily and share a common domain structure, the carbohydrate recognition domain (CRD), required for binding to specific carbohydrates (Drickamer 1993; Lobstein and Drickamer 1994). The CRD contains 18 highly conserved amino acid residues, including two disulfide bond folds formed by two cysteine residues (Day 1994; Kogelberg and Feizi 2001).

Over the past decade, cDNA cloning of DC and macrophage mRNA has led to the discovery of a number of receptors that exhibit high similarity to the CRD or exhibit the C-type lectin motif, which consists of three invariant cysteine residues at the C-terminus conserved among many CLRs. These receptors are referred to as C-type lectin-like (CTLL) receptors because most of them either do not possess an identified binding activity to carbohydrates, or bind to carbohydrates in a non-ionic-dependent manner.

CTLL receptors were initially sorted into two major types based on their structural features (Table I). This division remains a working cognitive framework that conceptualizes these receptors. Type I receptors are transmembrane polypeptides with multiple CRDs. This group includes the mannose receptor (MR, CD206), the phospholipase A2 receptor, DEC-205 (CD205), and Endo 180 (CD280), among others. By contrast, type II receptors are transmembrane polypeptides with a single CRD. This group includes dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN, CD209), langerin (CD207), macrophage galactose-type C-type lectin (MGL, CD301), the collectins, and the dendritic cell-associated C-type lectins 1 and 2 (dectin-1 and dectin-2), among others. More recently,

| Table I. C-Lectin-like receptors, their classifications, and ligands |
|-----------------------|----------------------|-----------------------|
| C-Lectin-like receptors | Type\(^a\) | Group\(^b\) | Pathogen-associated molecular patterns |
| Mannose receptor | I | VI | Mannose, fucose, N-acetylglucosamine |
| DC-SIGN | II | II | Mannin |
| Langerin | II | II | Mannose, fucose, N-acetylglucosaminylmannoside, mannoside |
| MGL | II | II | GalNAc |
| ASGP-R | II | II | Galactose |
| Collectins | III | III | Mannose, fucose, N-acetylglucosamine |
| Dectin-1 | II | V | β-glucan |
| Dectin-2 | II | VI | High-mannose oligosaccharide |

\(^{a}\)“Type” refers to carbohydrate recognition domain (CRD) type. Type I receptors contain multiple CRD, whereas type II receptors consist of a single CRD.

\(^{b}\)Group refers to domain organization and phylogeny as described by Zelensky and Gready.

The CLRs have been divided into 17 groups based on phylogeny and domain organization (Zelensky and Gready 2005) (Table I). CTLL receptors of all groups are thought to be involved in the recognition of pathogens and subsequent generation of the innate immune defense. The most studied CLRs are reviewed below, with emphasis on dectin-1 and dectin-2.

Mannose receptor

The mannose receptor (CD206) is a prototypical type I (group VI) transmembrane protein. It possesses eight extracellular tandem lectin-like carbohydrate recognition domains (CRD), a fibronectin type II repeat domain, a cysteine-rich domain, and a short cytoplasmic tail. The majority of the receptor is located within the intracellular endocytic pathway. The MR is expressed by macrophages, as well as by DC and other cells, and has been demonstrated to recognize several organisms, including C. albicans, C. neoformans, Pneumocystis carinii, and other pathogens such as bacteria and viruses (Klis et al. 2001; Taylor et al. 2005a, b; McKenzie et al. 2007). A putative role of MR in providing immunity against Mycobacterium tuberculosis has been noted by several authors (Chiappi et al. 2003; Jo 2008). The MR is able to bind mannose, fucose, N-acetylglucosamine, and glucose in a Ca\(^{++}\)-dependent manner. After carbohydrate recognition, the receptor mediates internalization of pathogens by phagocytosis, thereby leading to intracellular killing. Although the MR lacks classical signaling motifs in its cytoplasmic tail, it mediates a variety of cellular responses, including induction of NF-κB activation and the production of numerous defensive cytokines (Zhang et al. 2004). The MR has also been implicated in phagocytosis of fungi, although its exact role in this process has been debated (Le Cabec et al. 2005; Taylor et al. 2005a, b). Additionally, the MR may perform an immunosuppressive function in its ability to inhibit the production of inflammatory cytokines when certain fungal pathogens are recognized (Zhang et al. 2005).

DC-SIGN

One of the most extensively studied of the type II (group II) CTLL receptors is DC-SIGN, which is expressed primarily by DCs (Koppel et al. 2005). Structurally, this receptor possesses
an extracellular neck region of seven tandem-repeat sequences, a single CRD, and a cytoplasmic tail containing several internalization motifs. The make-up of the extracellular neck enables receptor multimerisation and specificity. DC-SIGN binds mannoside (high-mannose residues located internally within the glycan structure) in a Ca$^{++}$-dependent manner (Koppel et al. 2005). This activity enables the receptor to recognize a number of viruses, bacteria, and protozoa (Cambi et al. 2003; Serrano-Gomez et al. 2004, 2005). Additionally, DC-SIGN can induce endocytosis and has been proposed to mediate engulfment of certain fungi (Cambi et al. 2003; Serrano-Gomez et al. 2004; Koppel et al. 2005). The receptor has also been shown to bind mycobacterial products and to aid in the transport of these products to draining lymph nodes (Maeda et al. 2003; Taielleux et al. 2003). In fact, one cohort study of South Africans described an association between polymorphisms of the gene coding for DC-SIGN (CD209) and an increased susceptibility to tuberculosis (Barreiro et al. 2006). DC-SIGN also binds self-proteins and thereby facilitates cellular interactions of DC with T cells during antigen presentation and with endothelial cells during DC migration (Geijtenbeek et al. 2000a, 2000b). This receptor has can induce intracellular signaling via the Raf-kinase pathway, thereby modulating responses mediated by TLR and increasing expression of IL-10 (van Kooyk and Geijtenbeek 2003; Gringhuis et al. 2007). This immunosuppressive activity may be targeted by invasive pathogens (van Kooyk and Geijtenbeek 2003).

Langerin

Langerin (CD 207) is a group II transmembrane oligosaccharide receptor expressed by epidermal Langerhans cells, a subclass of dendritic cells that mediate immune responses in epithelia (Valladeau et al. 2000; Chatwell et al. 2007). Structurally, langerin consists of an intracellular region, a short transmembrane segment, and an extracellular neck region and CRD. Langerin displays two carbohydrate-binding sites in its extracellular domain. One is calcium-dependent and structurally conserved in the C-type lectin family; the other represents a novel, calcium-independent binding site (Chatwell et al. 2007). Langerin recycles between the plasma membrane and early endosomes and accumulates in Birbeck granules, an endosomal recycling compartment specific to Langerhans cells. It has been demonstrated to bind numerous monosaccharides, including mannose, fucose, and N-acetyl-glucosamine as well as oligosaccharides like mannan (Stambach and Taylor 2003). Langerin’s precise immunological role remains largely unknown. However, it has been implicated to play an important function in HIV-1 infection and defense (Kawamura et al. 2005; de Witte et al. 2007).

Macrophage glucose-type lectin

Macrophage glucose-type lectin (MGL) is unique among C-type lectins in its exclusive specificity for rare terminal GalNAc structures implicated in the homeostatic control of adaptive immunity (van Vliet et al. 2008). MGL is highly similar to the asialoglycoprotein receptor, the first member of the CLR family to be discovered and expressed exclusively by liver parenchymal cells (Ashwell and Harford 1982). Both ASGP-R and MGLs are classified as group II CLR s (Zelensky and Gready 2005). In contrast to ASGP-R, however, MGL is expressed by dendritic cells and macrophages and binds a specific carbohydrate (terminal GalNAc) in order to enhance phagocytosis and MHC class II antigen presentation (Valladeau et al. 2001; van Vliet et al. 2007). Although the precise role of MGL in immunologic defense remains to be elucidated, the specific nature of its binding site and the receptor’s participation in cellular signaling and antigen presentation hold promise for functional properties.

Collectins

The collectins are a unique group of five human and six mouse proteins secreted to form soluble multimeric complexes of receptors. Collectins constitute the group III CLR s and consist of monomers made up of four domains: a cysteine-rich N-terminus, a collagen-like domain, an alpha-helical coiled coil, and a C-terminal ligand-binding domain. As monomers, collectins recognize such carbohydrate ligands as mannose, fucose, and N-acetyl-glucosamine in a low-affinity, Ca$^{++}$-dependent fashion. However, the avidity of collectin binding is greatly enhanced through multimerization of the monomers into homotrimers that are subsequently linked via disulphide bonds to generate complex macromolecular structures (Holmskov et al. 2003). Three collectins, mannose-binding lectin (MBL) and surfactant proteins A (SP-A) and D (SP-D), have been demonstrated to be involved in a wide range of the innate immune response. MBL is synthesized primarily in the liver and recognizes C. albicans, A. fumigatus, and C. neoformans. Binding activates a protease cascade leading to opsonization of complement components on the microbial surface, thereby leading to immune clearance of the microorganism (Kilpatrick 2002; Holmskov et al. 2003). SP-A and SP-D are produced and secreted mainly in the lung and appear to recognize numerous pathogens, including Histoplasma capsulatum, C. albicans, and C. neoformans (Crouch and Wright 2001; Holmskov et al. 2003; Kishore et al. 2006). Through unknown mechanisms, SP-A and SP-D promote microbial agglutination and modulate phagocytosis, cytokine production, and the respiratory burst. Interestingly, the type of biological response elicited by the surfactant proteins appears to depend largely on the specific pathogen with which they interact (Rosseau et al. 1999; Willment and Brown 2007).

Dectin-1 and dectin-2

Structure

In the process of identifying genes expressed by DC but not by macrophages, we employed subtractive cDNA cloning between the XS DC and the J774 macrophage lines, leading us to discover several novel genes, two of which encode type II membrane-anchored proteins (Ariizumi, Shen, Shikano, Xu, et al. 2000), consisting of (from N-terminus) a cytoplasmic domain, a transmembrane domain, and an extracellular domain that is further divided into a stalk domain and a CRD-like domain (Figure 1A) (Ariizumi, Shen, Shikano, Ritter, et al. 2000). The two molecules were termed “Dendritic cell-associated C-type lectin-1 and -2,” or dectin-1 and dectin-2 because mRNA expression for the two genes was detected at high levels in XS and
bone marrow-derived DC lines and at significantly lower levels in macrophage lines (Ariizumi, Shen, Shikano, Xu, et al. 2000; Ariizumi, Shen, Shikano, Ritter, et al. 2000). The amino acid sequences of these two lectin receptors exhibit only moderate similarity (22% overall and 23% at the CRD-like domain), and the structure of their CRDs is also disparate (Ariizumi, Shen, Shikano, Ritter, et al. 2000). The CRD of dectin-1 contains 10 invariant amino acid residues conserved among many classical C-type lectins, with conservation of the three C-terminal cysteine residues that are implicated to form the fundamental structure required for carbohydrate binding (Spiess 1990; Ariizumi, Shen, Shikano, Xu, et al. 2000). By contrast, the CRD of dectin-2 contains 13 invariant residues and the EPN motif required for Ca++-dependent carbohydrate binding by the classical C-type lectins (Drickamer 1992; Weis et al. 1998; Ariizumi, Shen, Shikano, Ritter, et al. 2000). The structural features of the two dectins led to predictions that dectin-2 would be more likely to possess carbohydrate-binding activity and that carbohydrate ligands for the two receptors would be diverse.

**Expression**

Studies of mRNA expression revealed that dectin-1 and dectin-2 were both expressed constitutively at high levels by XS DC lines and by bone marrow-derived DC, but not by nonlymphoid cells (Fernandes et al. 1999; Ariizumi, Shen, Shikano, Ritter, et al. 2000; Yokota et al. 2001). More recently, researchers have employed specific monoclonal antibodies to show that dectin-1 is also expressed by many other cell types, including peritoneal and splenic macrophages, monocytes, neutrophils, and even a minor population of activated T cells (Brown 2006). In humans, dectin-1 is also expressed by B cells and eosinophils (Wilment et al. 2005). Expression of dectin-2 seems more restricted to DC, although it is expressed at low levels by tissue macrophages, blood monocytes, B cells, and neutrophils (Fernandes et al. 1999). Epidermal Langerhans cells appear selectively targeted for high-level expression of the dectin-2 receptor gene, with upregulation of expression exhibited after ultraviolet-B irradiation (Bonkobara et al. 2001, 2004, 2005). Macrophage expression of the receptor is markedly augmented by inflammatory stimuli in mice, thus raising the possibility that dectin-2 may serve as an activation marker for monocytes (Taylor et al. 2005a,b).

**Carbohydrate recognition**

Recent studies have established that dectin-1 recognizes β-glucan in a calcium-independent fashion and it is the major receptor for this carbohydrate on leukocytes (Brown et al. 2002; Brown 2006; Palma et al. 2006).

Although the structure of dectin-2 meets many of the requirements of C-type lectins that enable recognition of carbohydrates, characterization of the actual carbohydrate binding by this receptor has been somewhat difficult. This dilemma was resolved, in part, by demonstrating that dectin-2 possesses calcium-dependent mannose and fucose lectin activity with specificity for high-mannose structures. The affinity of dectin-2 is 10-fold lower than those of mannose receptor and DC-SIGN, however (McGreal et al. 2006). In sum, dectin-2 recognizes mannan-related carbohydrates in a Ca++-dependent manner but with low affinity.

Characterization of carbohydrate recognition by dectin-1 and dectin-2 has enabled studies of these receptors’ role in eliciting innate immunity toward pathogens that are known to possess appropriate ligands.

**Dectin-1 recognizes yeast**

*Candida albicans* is composed of β-glucan (60%), mannanprotein (35–40%), and chitin (1–5%). The outer layer of the yeast cell wall is enriched with mannanprotein, while the inner layer is composed of chitin and β-glucan. So, while β-glucan is the major component of the cell wall, it is normally hidden in the inner layer, and likely becomes accessible only when the yeast cell undergoes budding or dies (Kollar et al. 1997; Klis et al. 2001). Studies have demonstrated that soluble dectin-1 receptor does not bind all over live yeast cells, but rather binds almost exclusively at the budding site (Figure 2A) (Gantner et al. 2005; Gersuk et al. 2005; Hohl et al. 2005; Steele et al. 2005). This is consistent with a movement of β-glucan to the outer layer at the budding site as mannanprotein breakdown leads to ligand exposure. In response to environmental clues, such as host invasion, *C. albicans* converts from a unicellular yeast form to an invasive multicellular filamentous form. After filamentous transformation, β-glucan remains localized to the inner layer of the cell wall and thus escapes dectin-1 recognition (Heinsbroek et al. 2005). Thus, dectin-1 recognizes *C. albicans* yeast under given conditions but does not recognize *C. albicans* hyphae. Dectin-1 has also been shown to bind to a number of fungal organisms other than *C. albicans*, including *Aspergillus fumigatus*, *Pneumocystis carinii*, *Coccidioides posadasii*, *Microsporum audouinii*, and *Trychophyton rubrum* (Brown 2006).

**Dectin-2 recognizes hyphae**

While, as described above, dectin-2 has been shown to bind high-mannose carbohydrates with relatively low affinity, more research was required to identify specific microorganisms that the receptor recognizes. Fluorescently labeled dectin-2 receptor was employed to search for these microorganisms, and neither bacteria (*S. aureus*, group A streptococci, *P. aeruginosa*, or *E. coli*), nor *C. albicans* yeast bound to dectin-2. However, dectin-2 did bind *C. albicans* hyphae (Figure 2B) (Sato et al. 2006). This type of binding was also noted between dectin-2 and the filamentous, but not conidial, form of dermatophytes *M. audouinii* and *T. rubrum*. Binding was completely blocked by mannan, though not by β-glucan, thereby indicating that the putative hyphal ligand of dectin-2 is disparate from the conidial ligand of dectin-1.

The differential recognition of diverse forms of *C. albicans* by dectin receptors is also documented by selective binding of dectin gene-transfected RAW macrophage lines: dectin-1-RAW cells phagocytosed the yeast component of pseudo-hyphae, while dectin-2-RAW cells adhered only to the filamentous component (Figure 3). Moreover, dectin-1 recognizes the yeast form in a cation-independent manner, while dectin-2 recognizes the hyphal form in a cation-dependent manner.

Still, the exact structure of the dectin-2 hyphal ligand has not yet been identified. Numerous candidates can be eliminated based on an inability to block hyphal binding to dectin-2 (Figure 4). Since mannan completely blocks this binding, mannan-like or mannan-containing glycoproteins, glycolipids, or oligomannosides are the most likely dectin-2 ligands. Mannan alone is not likely the complete ligand, however, since both *C. albicans* yeast and hyphae both contain mannan on their
surfaces and yet dectin-2 binds hyphae but not yeast. Additionally, a relatively high concentration of mannann is required to inhibit binding of dectin-2 to hyphae. Clearly, further studies are needed to identify the dectin-2 ligand.

**Signaling and immune function of dectin-1**

Many immune receptors that transduce signals contain either the immunoreceptor tyrosine-based activation motif (ITAM) or the IT inhibitory motif (ITIM) in their intracellular domain. Following zymosan binding, dectin-1 is capable of inducing tyrosine phosphorylation of an ITAM-like sequence via Src family kinases, thereby providing docking sites for a signaling protein-termed spleen tyrosine kinase (Syk) (Rogers et al. 2005; Underhill et al. 2005). Syk had been thought to require two tandem YxxL sequences, but dectin-1 employs an atypical interaction with the Syk protein in that it possesses only one YxxL unit (Figure 1A) (Rogers et al. 2005; Underhill et al. 2005). Activated Syk leads to a wide variety of responses, including dectin-1-dependent phagocytosis, signaling for the production of cytokines and chemokines (such as IL-2 and IL-10), and activation of antigen presenting cells. Recently, the caspase recruitment domain-containing signaling protein CARD9 has been shown to operate downstream of Syk and to control the production of cytokines and chemokines (Gross et al. 2006; Hara et al. 2007; Ruland 2008). Although the specific mechanism is unknown, CARD9 directly couples Syk activation to the NF-κB pathway, thereby mediating Syk-dependent gene transcription and subsequent production of cytokines and chemokines (Gross et al. 2006; Hara et al. 2007). Recent findings suggest that dectin-1 induces Syk-independent signaling mediated by the serine-threonin kinase Raf-1, which eventually activates NF-κB. Raf-1 activation likely regulates expression of cytokines including IL-1β, IL-6, IL-10, and IL-12.

The in vivo significance of the dectin-1 signaling pathway has been analyzed by several groups using immunological analyses of mice where either the dectin-1 receptor itself or other components in its signaling pathway are rendered ineffective. Blockade of the receptor during intratracheal infection with Aspergillus fumigatus was shown to reduce inflammatory response and increase fungal burden (Steele et al. 2005). Another study found that mice with defective CARD9 displayed increased susceptibility to Candida albicans infection (Gross et al. 2006). The importance of CARD9 is highlighted by studies suggesting that mice deficient in this downstream signaling protein may be more susceptible to Candida infection than dectin-1 knockout mice (Saijo et al. 2007; Taylor et al. 2007).

Two groups disrupted the dectin-1 receptor gene directly. In one of these studies, dectin-1-deficient mice were generated by deleting exons 1–3 from the genome (Taylor et al. 2007). Dectin-1-deficient macrophages from these mice displayed impaired β-glucan recognition and were also deficient in inducing subsequent TNF-α expression, although the macrophages did retain normal TLR-induced responses. The mice themselves were highly susceptible to C. albicans. The other group’s data portrayed a disparate role of dectin-1 in anti-fungal immunity (Saijo et al. 2007). This group generated dectin-1-deficient mice via deletion of exons 1 and 2 from the genome. Dectin-1-deficient DC from these mice lacked the ability to induce cytokine expression by β-glucan but were able to induce cytokine expression normally by zymosan. Surprisingly, the dectin-1-deficient macrophages showed no defects in recognizing live C. albicans and the mice themselves showed no difference in susceptibility to Candida (but more susceptibility to P. carinii fungi) compared to control littermates. Although the two groups’ data agreed in demonstrating dectin-1 as a major receptor for β-glucan, the data are clearly discrepant in illustrating the role of dectin-1 in induction of anti-Candida immunity. This discrepancy may be accounted for by differences in the genetic background of deficient mice and/or by differing strains of C. albicans. Nevertheless, the true biological significance of dectin-1 in innate immunity remains to be defined.

**Signaling and immune function of dectin-2**

In contrast to dectin-1, dectin-2 lacks recognized signaling motifs and is incapable of inducing intracellular signaling on its own. However, our recent study demonstrated that dectin-2 associates with an adaptor receptor that induces tyrosine phosphorylation in order to mediate intracellular signaling (Sato et al. 2006). The Fc receptor γ (FcRγ) chain serves at the adaptor receptor through the interaction with dectin-2 between multiple intracellular domains. The FcRγ chain does not possess ligand specificity itself but is known to associate with many Ig superfamily receptors via the interaction between transmembrane domains, where a positively charged arginine residue is essential for this interaction. In contrast to Ig-like receptors, the association of dectin-2 with FcRγ was achieved via intracellular amino acid residues proximal to the transmembrane domain and not through transmembrane arginine (Sato et al. 2006).

Since the FcRγ chain contains an ITAM motif that links to Src family kinases, ligation of dectin-2 with hyphae (or surrogated ligand) leads to FcRγ phosphorylation, which leads to activation of NF-κB, the production of TNF-α and IL-1 receptor antagonist, and phagocytosis. That downstream events are blocked by PP2, an inhibitor of Src kinases, provides further evidence for the dectin-2-induced and FcRγ mediated signaling mechanism (Sato et al. 2006). Thus, dectin-2 is able to induce signal transduction leading to many cellular and molecular changes that unique and dissimilar to those initiated by the ligand interaction with dectin-1. Are signaling pathways induced by dectin-1 and dectin-2 different? Ligation of dectin-1 induces the Syk-dependent kinase pathway. Although yet to be formally demonstrated, dectin-2 is also likely to activate Syk kinase since the ITAM of FcRγ is known to activate Syk kinase. Dectin-1 and dectin-2 upregulate different sets of cytokines: the former induces IL-10 and IL-12, whereas the latter does not; dectin-2 induces IL-1 receptor antagonist (IL-1ra) and TNF-α, whereas dectin-1 does not. These differences may be due to Raf-1 signaling.

**Differentiation among forms of C. albicans by various PRRs**

After host invasion, C. albicans switches from the yeast form to the filamentous; this transformation has been implicated to contribute to virulence. In fact, mutant Candida rendered unable to transform are considerably less virulent than the wild-type cells (Lo et al. 1997; Laprade et al. 2002). Mechanisms of virulence employed by wild-type C. albicans include inhibition of DC induction of IL-12 expression, a cytokine that plays a critical role in the differentiation of naïve T cells into Th1 cells...
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(Tang et al. 2004). Moreover, phagocytosis of Candida hyphae by DC enhances the production of IL-4, which also prevents differentiation of T cells into Th1-type cells (d’Ostiani et al. 2000). Other studies have demonstrated that C. albicans mutants defective in mannosylation exhibit a markedly reduced ability to stimulate cytokine expression by macrophages; polysaccharides are indeed central to anti-Candida immunity (Netea et al. 2006).

During the course of C. albicans infection, DC are likely to sense the yeast-to-filamentous switch by using dectin-1 and dectin-2 receptors. In the immediate phase of infection, DC recognize C. albicans yeast by TLR4, mannose receptor, DC-SIGN (all binding to mannosyl residues in the outer layer mannanprotein), and by integrin CR3. Dendritic cells may employ dectin-1 to detect budding C. albicans yeast. The dectin-1 signaling pathway may facilitate phagocytosis, and activate the production of specific cytokines and chemokines, such as IL-2 and IL-10, via MAPK and quickly reconfigures its cell wall structure and components, one of which may be recognized by dectin-2 along with other receptors (mannose receptor, DC-SIGN, and integrin CR3). The dectin-2 signaling pathway may then activate a disparate mechanism of immune defense, including upregulated secretion of TNFα and IL-1 receptor antagonist. Although many receptors are involved in the recognition of these two forms of C. albicans, dectin-1 and dectin-2 are unique in providing a form-specific recognition: dectin-1 recognizes yeast through binding of β-glucan, while dectin-2 recognizes the hyphae through binding to a high-mannose-oligosaccharide that remains unidentified. Using dectin receptors, DC may distinguish yeast from hyphae, after which diverse signaling pathways may adjust DC function to better combat each of these forms.

Conclusion
Polysaccharide and lipoprotein constituent the molecular pattern specific to a given microbial species. These PAMPs are recognized by a variety of receptors within the innate immune system, including TLRs, NLRs, and CLRss. Interplay among these receptors and their signaling mechanisms likely shapes the innate and adaptive immune response. The ultimate outcome of this collaboration is dictated by the molecular pattern specific to the pathogen: DC employ diverse and overlapping receptors to sense different types of species, to detect different virulence states of individual pathogens, and to induce intracellular signaling pathways that tailor the host immune response to the specific state of a particular microorganism.

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None declared.

Abbreviations
APC, antigen-presenting cells; CLR, C-type lectin receptors; CRD, carbohydrate recognition domain; DC, dendritic cells; IRF3, interferon-regulatory factor 3; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, IT inhibitory motif; NLR, NOD-like receptors; NO, nitric oxide; NOD, nucleotide oligomerization domain; PAMPs, pathogen-associated molecular patterns; PRR, pattern recognition receptors; Syk, spleen tyrosine kinase; TLR, toll-like receptors.

References


