Dendritic Cell Interaction with Candida albicans Critically Depends on N-Linked Mannan

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The fungus Candida albicans is the most common cause of mycotic infections in immunocompromised hosts. Little is known about the initial interactions between Candida and immune cell receptors, because a detailed characterization at the structural level is lacking. Antigen-presenting dendritic cells (DCs), strategically located at mucosal surfaces and in the skin, may play an important role in anti-Candida protective immunity. However, the contribution of the various Candida-associated molecular patterns and their counter-receptors to DC function remains unknown. Here, we demonstrate that two C-type lectins, DC-SIGN and the macrophage mannose receptor, specifically mediate C. albicans binding and internalization by human DCs. Moreover, by combining a range of C. albicans glycosylation mutants with receptor-specific blocking and cytokine production assays, we determined that N-linked mannann but not O-linked or phosphomannnn is the fungal carbohydrate structure specifically recognized by both C-type lectins on human DCs and directly influences the production of the proinflammatory cytokine IL-6. Better insight in the carbohydrate recognition profile of C-type lectins will ultimately provide relevant information for the development of new drugs targeting specific fungal cell wall antigens.

The fungus Candida is the most common cause of opportunistic mycotic infections in severely immunocompromised hosts, such as surgical, cancer, and transplant patients, worldwide. During infection, the transition from simple yeast form to multicellular filaments (hyphae) plays a fundamental role in evading the host immune defense. C. albicans remains the most common Candida species in human pathology, but the prevalence of other species including C. glabrata, C. parapsilosis, and C. dubliniensis, remains significant. The stimulation of proinflammatory cytokines and the subsequent activation of antifungal host defense depend on specific recognition of the invading fungus. Cells of the innate immune system recognize pathogens by identifying conserved microbial structures, called pathogen-associated molecular patterns (PAMPs), which are structurally distinct from molecules expressed on mammalian cells. The Candida cell wall is almost exclusively composed of glycans. Glycans are synthesized as polymers of three types of monosaccharides: α-glucose, which forms β-(1,3) and β-(1,6) glucan, N-acetyl-α-glucosamine, which forms chitin, and β-mannose, giving rise to mannan (5). The outer layer of the C. albicans cell wall is enriched in mannan, representing up to 30–40% of the cell wall dry weight (6), while chitin, β-(1,3)- and β-(1,6)-glucan are more prominent in the inner layer. In addition, the interplay between C. albicans glycans and the host induces modifications in C. albicans morphogenesis that are associated with changes in cell wall composition (7–9). Little is known about the initial interactions between Candida PAMPs and immune cell receptors, because a detailed characterization at the structural level is lacking.

The importance of detailed knowledge of the fungal PAMP repertoire and activity is also underscored by the development of more and more fungal glycans-based vaccines (10–13). Studies by Cutler and co-workers (14, 15) showed that monoclonal antibodies specifically directed against Candida mannan are able to enhance resistance to C. albicans infection in mice. Similarly, efforts are aimed at the development of synthetic glycoconjugates based on fungal glycan structures that provide protection against candidiasis (16). A recent study also showed that yeast-derived mannosylation of recombinant tumor antigens...
enhanced immunogenicity and indeed efficiently stimulated T cell responses (17).

Whereas most *C. albicans* organisms are cleared by neutrophils and macrophages (18–20), the strategic location of antigen-presenting dendritic cells (DC) at mucosal surfaces and in the skin, the sites of *Candida* invasion, positions DCs in the first line of defense to obtain protective immunity. Despite this, the relative contribution of the various *Candida* PAMPs and their receptors to binding and cytokine stimulation by DCs remains unknown.

DCs express several pattern-recognition receptors (PRRs) that specifically recognize distinct PAMPs displayed on fungal surfaces including C-type lectin receptors (CLRs) (21) and Toll-like receptors (TLRs) (22). The macrophage mannose receptor (MMR), member of the CLR family, has been shown to recognize *C. albicans* on DCs (23), and we previously demonstrated that the DC-associated CLR DC-SIGN (CD209) is also involved in binding and internalization of *C. albicans* by human DCs (24).

Despite the progress in understanding the interaction of some of the fungal PAMPs with PRRs expressed on DCs, there is no integrated view of the mechanism(s) by which DCs sense *C. albicans* PAMPs. We recently showed that recognition of *C. albicans* by monocytes/macrophages is a multiple step process involving several PRRs recognizing the various layers of the outer portion of the fungal cell wall (25).

Here, by combining a range of *C. albicans* isogenic glycosylation mutants with receptor-specific blocking and cytokine production assays, we determined the structure of the fungal mannan that is specifically recognized by the CLRs on human DCs and can directly influence the production of the pro-inflammatory cytokine IL-6. Our results indicate that different mannann structures may skew the cytokine profile of DCs and differentially modulate the immune response. Therefore, better insight in the carbohydrate recognition profile of CLRs will ultimately provide relevant information for the development of new drugs targeting specific fungal cell wall antigens.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Fluorescein isothiocyanate (FITC) was from Sigma. Recombinant gp120, mAb 1748 anti-DC-SIGN, and polyclonal goat-anti-mouse IgG2b were from R&D Systems Europe (Oxon, UK). ICAM-3Fc was produced as already described (24). To test the effect of various reagents on ligand binding the following concentrations were used: different carbohydrates 150 μg/ml (unless otherwise indicated), EGTA 2 mM, isotype control (mouse IgG1), AZN-D1 anti-DC-SIGN, anti-MMR, anti-Dectin-1, anti-DCIR, and anti-DEC-205 (30 μg/ml). Incubations were performed in 20 mM Tris, pH 8.0, 150 mM NaCl, 1 mM CaCl₂, 2 mM MgCl₂, and 1% bovine serum albumin, as already published (24). FITC-labeled *Candida* was added in a cell/yeast ratio of 1:5. After 30 min of incubation at 37 °C, cell-yeast conjugates were analyzed by flow cytometry.

**Phagocytosis**—Immature DCs (5 × 10⁵) were allowed to adhere onto fibronectin and subsequently incubated with FITC-labeled *C. albicans* or *C. dubliniensis* yeast cells (2.5 × 10⁶) or FITC-labeled *C. albicans* CAI-4 wild-type and mannosylation mutants (2.5 × 10⁶) for 60 min at 37 °C. At the end of the incubation period, the samples were fixed in 4% PFA, permeabilized in Triton X-100, and labeled for DC-SIGN and/or the MMR using specific mAb and isotype-specific fluorescent
DC-SIGN Specifically Interacts with Candida-derived Mannan—The Candida cell wall is primarily comprised of mannan and by a lesser content of glucan and chitin (5). DC-SIGN is known to bind high mannose moieties (47). To determine the PAMP structures specifically recognized by DC-SIGN, we previously demonstrated that DC-SIGN binds and internalizes C. albicans (24). Because several distinct Candida species are commonly found in patients with candidiasis (2, 3), we analyzed the capacity of DC-SIGN to recognize and bind a range of different Candida species (Fig. 1A). K562 cells stably expressing DC-SIGN (K-DC-SIGN) strongly bound C. albicans, C. dubliniensis, and C. glabrata, but only weakly interacted with C. parapsilosis. In addition, DC-SIGN bound S. cerevisiae-derived zymosan particles. Anti-DC-SIGN mAb and the Ca2+-chelating agent EGTA effectively inhibited the interactions demonstrating the specificity for DC-SIGN. DC-SIGN contributes to the binding and internalization of C. albicans by DCs (24). In Fig. 1B, we compare the binding of DCs isolated from different donors to C. albicans and C. dubliniensis and show that similarly to C. albicans, a significant percentage of the binding to C. dubliniensis was also mediated by DC-SIGN. Moreover, both C. albicans and C. dubliniensis yeast cells were found in DC-SIGN-enriched vesicles (Fig. 1C). Similar results were obtained when zymosan was used (data not shown). These results demonstrate that although the affinity of DC-SIGN varies among Candida species, in DCs DC-SIGN is involved in the uptake of all Candida species tested.

**RESULTS**

**DC-SIGN Recognizes Several Species of Candida**—We previously demonstrated that DC-SIGN binds and internalizes C. albicans (24). The percentage of cells that bound fluorescent beads was determined by flow cytometry. In inhibition studies, the assay was performed in the presence of the inhibitors, as described above for the Candida binding studies.

**NMR Spectroscopy**—The proton and carbon-13 NMR spectra were collected on a JEOL Eclipse+ 600 NMR spectrometer in 5-mm OD NMR tubes at 70 °C in D2O. Internal chemical shift reference was provided by trimethylsilyl-2,2,3,3-d4-propionic acid (TSP) (43–45) at 0.0 and −2.78 ppm for 1H and 13C spectra, respectively. Individual solutions of SC-mannan and CA-mannan were prepared at concentrations of 24 and 15 mg per 1 ml of solvent, respectively.

**GPC/MALLS Methodology**—The molecular weight distributions of the mannans were established by gel permeation chromatography (GPC) with online multi-angle laser light scattering photometry (MALLS) as described previously (46).

**Stimulation of IL-6 Production in Human DCs**—Samples of 3 × 10^5 immature DCs in 300-μl volume were added to round-bottomed 48-well plates (Greiner Bio-One) and incubated with the various strains of C. albicans (ratio DCs/Candida = 1:10). After incubation for 6 h at 37 °C, the DCs/C. albicans cell suspensions were centrifuged, and the supernatants were collected and stored at −70 °C until assayed. IL-6 concentration was measured by commercial ELISA kits (Sanquin), as already reported (25).
with both *C. albicans* and *C. dubliniensis* were not affected by the pretreatment with SC-mannan (Fig. 2A).

To further investigate whether the interaction between DC-SIGN and *Candida* occurs at the carbohydrate recognition domain (CRD) of the receptor, two mutants of DC-SIGN, each carrying a specific point mutation known to affect binding, were compared with the wild-type receptor for their ability to bind to the fungus. The amino acid residue Val351 has been shown to be essential for DC-SIGN-mediated binding, because its mutation to Gly abrogated the interaction with ICAM-3 (49) and LewisX (50). Furthermore, it was reported that mutating the Ca$^{2+}$-chelating residue Asp324 into Ala resulted in complete loss of ligand binding (49). The observation that the V351G mutant of DC-SIGN binds to *Candida* much less efficiently than wild type and that the E324A mutant had totally lost its capacity to interact with the fungus (Fig. 2B) demon-

![Figure 2](https://www.jbc.org/content/283/29/20593/F2)

**FIGURE 2.** DC-SIGN specifically recognizes mannan of *C. albicans* cell wall. A, K-DC-SIGN cells were incubated with different ligands in the presence or absence of anti-DC-SIGN blocking agents, as described under “Experimental Procedures.” ICAM-3- and gp120-beads indicate fluorescent beads ($1 \mu m$ Ø) coated with soluble ICAM-3 and gp120, respectively. The percentage of binding was calculated by flow cytometry and indicates the proportion of cells that became fluorescent upon interactions with either fluorescent beads or FITC-labeled fungi. B, K562 cells stably expressing similar levels of either DC-SIGN wild type or V351G- or E324A-DC-SIGN mutant were incubated with FITC-labeled *C. albicans* in a ratio of 1:5, in the presence or absence of blocking agents, as described under “Experimental Procedures.” C, K-DC-SIGN cells were incubated with FITC-labeled *C. albicans* in a ratio of 1:5, in the presence or absence of purified fungal cell wall glycans. CA-mannan 50 and CA-mannan 100 indicate a concentration of 50 and 100 $\mu g/ml$, respectively. All other glycans were used at a concentration of 150 $\mu g/ml$, as described under “Experimental Procedures.” Basal binding (none) is set as 100%. Data are presented as means ± S.D. Results are pooled from three independent experiments. D, DCs were incubated with *C. albicans* FITC-yeast cells in the presence or absence of purified glycans. Basal binding (none) is set as 100%. Data are presented as means ± S.D. Results are pooled from three independent experiments. E, DCs were incubated with *C. albicans* FITC-yeast cells in the presence of different CLR-blocking mAbs (30 $\mu g/ml$). Basal binding (none) is set as 100%. Data are presented as means ± S.D. Results are pooled from three independent experiments. *, $p < 0.01$ versus basal binding, as determined by the two sample t-test.
strates that the interaction between DC-SIGN and *C. dubliniensis* is specifically mediated by the CRD. It has to be noted that the expression level of these mutated forms of DC-SIGN is comparable to the wild-type (supplemental Fig. S1).

To get further insight into the specificity of DC-SIGN recognizing mannan structures from fungi, we analyzed several purified fungal cell wall carbohydrates (supplemental Table S1) for their capacity to inhibit this interaction. Strikingly, CA-mannan was the only fungal cell wall carbohydrate that significantly inhibited K-DC-SIGN binding to *C. albicans* in a concentration-dependent manner (Fig. 2C). The high specificity of this recognition was further demonstrated by the observation that CA-mannan but not SC-mannan was able to inhibit binding of *C. dubliniensis* to K-DC-SIGN (supplemental Fig. S1A). The inhibitory effect of SC-mannan on DC-SIGN binding to zymosan (Fig. 2A) suggests that SC-mannan may better represent the structure of the zymosan mannan rather than the mannan structure exposed at the cell wall of *Candida*.

Similarly, we demonstrated that CA-mannan was the only fungal cell wall-derived carbohydrate tested that specifically and significantly abrogated binding of DCs to *C. albicans* (Fig. 2D). The blockade of binding observed after pretreatment of DCs with CA-mannan but not with other carbohydrates also shows that no significant role is played by glucan receptors on human DCs in binding *C. albicans*. This notion is further supported by the finding that blocking of DC binding to *C. dubliniensis* was also detected only in the presence of CA-mannan (supplemental Fig. S1B). In agreement with these observations, when a panel of DC-associated CLRAs was blocked, only inhibition of MMR and DC-SIGN significantly affected the recognition of *Candida* by DCs (Fig. 2E). These data clearly indicate that the PAMP specifically recognized by DCs in the interactions with *Candida* is mannan.

**DC-SIGN Recognizes Structural Differences in Fungal N-Mannan—**To reveal the differences between CA- and SC-mannan, we next performed a comparative structural analysis by nuclear magnetic resonance (NMR) (supplemental Fig. S2). All fungal mannans consist of a backbone of α-(1,6)-linked mannosyl repeat units with side chains of α- or β-(1,2)-linked mannosyl repeat units connected to the backbone by a α-(1,2)-linkage as well as phosphate di-mannosyl esters and α-(1,3)-linked mannosyl units at the end of the side chains (51). The α-anomer glycosidic linkage configuration was the major conformation found with a low level of β-anomer glycosidic linkage configuration. Structural characterization of the mannans indicated that CA-mannan better resembles the native mannan from the cell wall of living *Candida* cells than SC-mannan (supplemental Fig. S2). Molecular weights of the mannans were established by GPC/MALLS analysis. The GPC-determined molecular weight for SC-mannan was 3.7 \times 10^4 \text{ g/mol}, while for CA-mannan was 4.9 \times 10^6 \text{ g/mol}. This molecular weight difference is supported in the resonance line widths observed in the C-13 NMR spectra (supplemental Fig. S2C). Schematic representations of the structural features derived from NMR and GPC/MALLS analysis are given in Fig. 3A (see also supplemental Fig. S2D for a detailed structure). This comparative NMR analysis clearly confirmed that the SC- and CA-mannan used in our studies significantly differ in terms of size and branching, suggesting that these specific structural differences between the two mannans could account for differences in affinity for the receptor and therefore in blocking capacity.

Subsequently, to better understand which structural elements of the fungal mannan are essential for the interaction between *Candida* and DC-SIGN, we exploited the well-defined isogenic mutants of *C. albicans* depleted in specific mannan structures (25). In Fig. 3B, the N- and O-mannan structures of the wild-type *Candida* strain and the isogenic mutants are displayed. The och1 mutant lacks branched outer N-linked mannosyl chains (38), while the pmr1 mutant has defects both in N- and O-linked mannosylation (39). The mnn4 mutant only lacks phosphomannan linked to the N-mannan (40), and the double mutant mnt1/mnt2 has intact N-mannan but lacks the 4 terminal O-linked α-(1,2)-mannosyl residues (41).

We used these mutants to analyze the interaction between K-DC-SIGN and *Candida* mannan (Fig. 3C). Binding to DC-SIGN was severely reduced in the pmr1 mutant as well as in the och1 strain. In contrast, the absence of mannosylphosphate (mnn4) or O-linked mannan (mnt1/mnt2) had no effect on the interaction between DC-SIGN and *Candida*. Similarly, when *C. dubliniensis* glycosylation mutant och11 was used, no binding of K-DC-SIGN was detected (supplemental Fig. 4A), indicating that the N-linked mannan is the general structure recognized by DC-SIGN in all *Candida* species. These data unequivocally demonstrate that N-mannan is the only structure required for the recognition of *Candida* by DC-SIGN and that no O-linked or phosphomannan structures are recognized.

To further define the N-mannan epitope responsible for this interaction, *C. albicans* serotype B was used. Serotype B specifically lacks the terminating β-1,2-linked mannose units in the acid-stable region, but still has the β-1,2 mannosyl attached to the phosphomannan (52). As shown in Fig. 3D, DC-SIGN is able to bind to serotype B *Candida*, suggesting that the terminating β-1,2 mannosyl units are not required for binding. K-DC-SIGN cells were also able to bind to serotype B mnn4Δ mutant, which lacks all β-1,2 mannose residues (36). This further indicates that no β-1,2 mannosyl is involved in the interaction of DC-SIGN with *Candida* (data not shown).

**N-Mannosylation Mediates Binding, Phagocytosis, and Cytokine Production by Dendritic Cells—**Next, exploiting the same series of *Candida* glycosylation mutants, we determined which mannan structures specifically were involved in recognition of *Candida* by DCs expressing both DC-SIGN and MMR (Fig. 4A). The mannan structures recognized by DC-SIGN on the transfected K562 cell line were also required to mediate the interaction between the DCs and *Candida*. Wild-type N-mannan, with or without phosphomannan, is therefore essential for interaction of *C. albicans* with DCs. Similarly to what observed for K-DC-SIGN cells, DCs also bind to *C. albicans* serotype B wild-type as well as Δmnn4 (supplemental Fig. S5), indicating that no terminating β-1,2-mannose residues are involved in the binding of *Candida* to DCs. Finally, binding of DCs to *C. dubliniensis* is also depending on the presence of the N-linked mannan.
nan, as shown by the lack of interaction between DCs and C. dubliniensis och1 mutant (supplemental Fig. S4B), further supporting the important role played by N-linked mannan in the binding of Candida to DCs. Subsequently, we analyzed the individual contribution of DC-SIGN and MMR to the binding of DCs to the Candida wild-type, mnt1/mnt2, and mnn4 mutants (Fig. 4B). The partial block observed in the presence of the anti-DC-SIGN blocking mAb is in agreement with previous observations, where MMR and DC-SIGN were responsible for ~70 and ~30% of the binding to Candida, respectively (24). Moreover, similarly to what was observed upon preincubation with CA-mannan, inhibition of both MMR and DC-SIGN
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**FIGURE 4.** N-mannosylation mediates binding, phagocytosis, and IL-6 production by DCs.  

A, DCs were incubated with FITC-labeled *C. albicans* wild-type and mutated strains for 30 min at 37 °C, as described under “Experimental Procedures.” Data are presented as means ± S.D. Results are pooled from three independent experiments with six different donors.  

B, DCs were incubated with FITC-labeled *C. albicans* wild-type and mutated strains in the presence or absence of blocking mAb for 30 min at 37 °C, as described under “Experimental Procedures.” The percentage of binding was calculated by flow cytometry: basal binding was set as 100%. Data are presented as means ± S.D. of a representative experiment out of 5 performed in duplicate.  

C, DCs were allowed to adhere onto fibronectin-coated glass coverslips for 30 min at 37 °C and subsequently incubated with FITC-labeled *C. albicans* wild-type and mutated strains (green) for 90 min at 37 °C to allow phagocytosis. After washing of unbound *Candida*, fixation, and permeabilization, DCs were fluorescently labeled with mAbs specific for DC-SIGN (red) and the MMR (blue). Scale bars, 10 μm.  

D, DCs were stimulated with the various *C. albicans* strains: the wild-type strain CAI-4, the pmr1 mutant defective in both N- and O-mannosylation, the och1 null mutant lacking the branched outer N-linked mannosyl chain, the mnt1/mnt2 mutant lacking only the 4 terminal O-linked α1,2-mannosyl residues, and the mnn4 mutant defective in phosphomannan. After incubation for 6 h at 37 °C, supernatants were collected, and IL-6 expression was determined by ELISA. Results (mean ± S.D.) are pooled data from three separate experiments with three different donors. *, p < 0.01 versus wild-type CAI-4, as determined by the two sample t-test.
almost completely abrogated the interaction of the various mutant strains with DCs (Fig. 4B). In Fig. 4C, we show that in addition to binding, DCs also phagocytose _C. albicans_ mutants, and that both MMR and DC-SIGN are found around the _Candida_ containing phagosomes. While the och1 and pmr1 mutants lacking N-linked mannann chains were not phagocytosed, mutants with an intact N-mannan but with defects in O-mannosylation and phosphomannan biosynthesis—such as mnt1/mnt2 and mnn4—were readily bound and internalized. Three-dimensional Z-sectioning of DCs in the confocal microscope unequivocally proved that the _Candida_ yeast cells were not only bound but indeed ingested by DCs.

Finally, we investigated the role of N- and O-linked mannosylation in stimulating cytokine production in human DCs by _C. albicans_ by comparing the production of the prototype pro-inflammatory cytokine IL-6, induced after 6 h of exposure to the various mannann defective mutants. The absence of O-linked mannosylation (mnt1/mnt2, mnn4) did not have any significant effect on IL-6 expression, whereas the mutants defective in N-linked mannosyl residues (och1, pmr1) produced only very low levels of cytokine (Fig. 4D). The same pattern was observed after 20 h of incubation (data not shown). Altogether these results clearly demonstrate that the presence of N-linked mannosyl residues is essential for interaction, recognition, and internalization of _Candida_ yeast cells as well as IL-6 production by DCs.

**DISCUSSION**

Disseminated infections with _C. albicans_ cause significant morbidity and mortality among immunocompromised individuals, such as HIV patients, transplant recipients, and cancer patients (1). Therefore, a better understanding of the specific receptors involved in the interactions between invading fungi and immune cells is necessary.

We demonstrated previously that DCs bind and internalize _C. albicans_ through the C-type lectin DC-SIGN (24). Here we show that besides _C. albicans_, DC-SIGN also binds to yeast cells of a range of _Candida_ species as well as to zymosan particles. Furthermore, we determined that the PAMP bound by DC-SIGN on _Candida_ cell wall is mannann, and that N-linked mannosyl residues are essential for this interaction. Finally, we demonstrate that the N-mannosylation is specifically required for the binding, phagocytosis, and immune sensing of _C. albicans_ by the DCs.

Interactions between host and fungal pathogens occur at the level of the cell wall (7), which is composed of glycoproteins embedded within a polysaccharide matrix. Therefore, carbohydrates are the immediate point of interaction with host tissues. A detailed characterization of these initial interactions at a molecular level has been lacking. The findings in this report demonstrate that the interaction between DC-SIGN and _C. albicans_ is mediated by mannan rather than other fungal cell wall components such as glucan, chitin, or chitin-glucan complex. However, the SC-mannan, purified from _S. cerevisiae_ cell wall, was not able to block the interaction between DC-SIGN and _Candida_, whereas CA-mannan, purified from _C. albicans_, could specifically inhibit binding of this fungus to DC-SIGN. This observation together with structural data obtained by the NMR and GPC-MALLS analysis indicate that SC-mannan and CA-mannan differ in their average structures, with SC-mannan being a smaller polymer without a phosphate diester linkage, while the CA-mannan is a larger polymer containing the phosphate diester linkage. SC-mannan is isolated by a procedure involving acetylation, which selectively cleaves α-(1,6)-linkages in the backbone, while CA-mannan isolation did not involve acetylation (53, 54). Therefore, different isolation protocols could be responsible for the different structural properties.

However, the recent work of Shibata _et al._ (55) shows the chemical structure of the cell-well mannan of _C. albicans_ after acetylation that is overall similar to the CA-mannan structure used in this study. These differences in mannan structure may account for the observed differences in receptor interaction and binding efficiency.

To investigate the role of cell wall mannosyl groups in the recognition of _C. albicans_ yeast cells by DCs and DC-SIGN in particular, specific isogenic mutant strains of _C. albicans_ were used with specific defects in the mannosylation of cell wall proteins. Gross defects in both N-linked and O-linked mannosylation were investigated using _C. albicans_ pmr1 mutant (39). The role of O-linked mannosylation was assessed using the mnt1/mnt2 mutant that lacks two α1,2-mannosyl transferases required for the addition of mannostere residues to a linear oligomannoside (41), while the role of phosphomannan was studied in the mnn4 mutant (40). Finally, the importance of N-linked mannosylation was indicated in experiments using the och1 mutant strain, which is unable to synthesize the branched outer mannan chains (38). We demonstrated that for the interaction with _C. albicans_, DC-SIGN required wild type N-linked mannan, but was not dependent on the presence of phosphomannan, or β-1,2 mannose or O-linked mannosyl residues. Thus altogether our binding studies indicate that the epitope necessary for binding of _Candida_ to DC-SIGN—expressing cells must be within the α-1,2 branched mannosyl residues present in the acid-stable part of the N-linked mannan (Fig. 5).

The binding of _C. albicans_ specifically involves the CRD of the lectin receptor, since mutations that altered amino acid residues known to be essential for ligand binding impaired the binding of DC-SIGN to _Candida_. Moreover, the V351G mutant does not bind ICAM-3 (49) nor LewisX (50), but does bind gp120 (49). Therefore, its lower but detectable binding to _C. albicans_ might suggest that the binding site of DC-SIGN responsible for the interaction with CA-mannan partially overlaps with that of gp120.

On the other hand, preincubation of K-DC-SIGN cells with well-defined N-linked oligosaccharides did not affect the binding to _C. albicans_ (supplemental Fig. S6), suggesting that the complex CA-mannan moiety might not bind to DC-SIGN as conventional high mannose oligosaccharides. The binding of DC-SIGN to synthetic fragments of high mannose oligosaccharides has been shown to occur via multiple modes that may involve different amino acids and further enhance the receptor avidity (56, 57). Moreover, the intrinsic oligomerization of DC-SIGN (58) and its tendency to form nanoclusters (59) are likely to provide additional regulation levels for the interaction of DC-SIGN with this type of fungal mannan arrays.
DCs bind to C. albicans via two CLRs, MMR and DC-SIGN. Interestingly, although the MMR recognizes single terminal mannosese residues and DC-SIGN interacts with more complex mannosese residues in specific conformations, the same mannosylation mutations that hamper DC-SIGN binding to C. albicans ablack the O-linked mannan (mnt1/mnt2), to C. albicans lacking the phosphomannose (mmn4) and to C. albicans lacking the terminating β-1,2 mannosese residues (serotype B wild-type as well as Δmmn4), indicating that these moieties do not represent the essential binding epitope. On the contrary, DC-SIGN-expressing cells are unable to bind to C. albicans that has a dramatic reduction (pmr1) or a complete loss (och1) of the branched acid-stable N-linked mannan part. Mannosyl residues not essential for binding are shown in gray.

This study represents the first comprehensive attempt to understand the mechanism of C. albicans recognition by human DCs. We conclude that recognition of C. albicans lacking the terminating mannosylation mutations that hamper DC-SIGN binding to C. albicans is via two CLRs, MMR and DC-SIGN. Interestingly, although the MMR recognizes single terminal manno- spite of the fact that DCs are unable to bind to C. albicans that has a dramatic reduction (pmr1) or a complete loss (och1) of the branched acid-stable N-linked mannan part. Mannosyl residues not essential for binding are shown in gray.

To understand how CLRs expressed on DCs discriminate between endogenous and pathogen-associated antigens, detailed knowledge of the specific carbohydrate structures recognized is essential. Especially the use of mutants that differ in their carbohydrate make-up are a valuable tool because we test an intact pathogen rather than isolated structures. This will allow us to get better insight in how the integrated signals from both CLRs and TLRs act on the immune system and how carbohydrate recognition profile analyses can ultimately lead to the development of new drugs targeting specific microbial carbohydrate antigens.

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REFERENCES

1. McNeil, M. M., Nash, S. L., Hajjeh, R. A., Phelan, M. A., Conn, L. A., Pilkeytis, B. D., and Warnock, D. W. (2001) Clin. Infect. Dis. 33, 641–647
2. Abi-Said, D., Anaissie, E., Uzun, O., Raad, I., Pinzowski, H., and Vartavarian, S. (1997) Clin. Infect. Dis. 24, 1122–1128
3. Coleman, D. C., Sullivan, D. J., Bennett, D. E., Moran, G. P., Barry, H. J., and Shanley, D. B. (1997) Annu. Rev. Immunol. 20, 197–216
4. Janeway, C. A., Jr., and Medzhitov, R. (2002) Annu. Rev. Immunol. 20, 251–255
5. Musauka, J. (2004) Clin. Microbiol. Rev. 17, 281–310
6. Klin, F. M., de Groot, P., and Hellingwerf, K. (2001) Med. Mycol. 39, Suppl. 1, 1–8
7. Calderone, R. A. (1993) Trends Microbiol. 1, 55–58
8. Choi, W., Yoo, Y. J., Kim, M., Shin, D., and Jeon, H. B. (2003) Yeast 20, 1053–1060
9. Lowman, D. W., Ferguson, D. A., and Williams, D. L. (2003) Carbohydr. Res. 338, 1491–1496
10. Bystrický, S., Paulovicová, E., and Machová, E. (2003) Immunol. Lett. 85, 251–255
11. Rutler, J. E. (2005) Curr. Mol. Med. 5, 383–392
12. Dromer, F., Chevalier, R., Sendid, B., Improvisi, L., Jouault, T., Robert, R., Mallet, J. M., and Poulin, D. (2002) Antimicrob. Agents Chemother. 46, 3869–3876
13. Torosantucci, A., Bromuro, C., Chiani, P., De Bernardis, F., Berti, F., Galli, C., Norelli, F., Bellucci, C., Polonelli, L., Costantino, P., Rappuoli, R., and Cassone, A. (2005) J. Exp. Med. 202, 597–606
14. Han, Y., Morrison, R. P., and Rutler, J. E. (1998) Infect. Immun. 66, 5771–5776
15. Han, Y., Riedesel, M. H., and Rutler, J. E. (2000) Infect. Immun. 68, 1649–1654
16. Wu, X., and Bundle, D. R. (2005) J. Org. Chem. 70, 7381–7388
17. Lam, J. S., Mansour, M. K., Specht, C. A., and Levitz, S. M. (2005) J. Immunol. 175, 7496–7503
18. Marodi, L., Korchak, H. M., and Johnston, R. B., Jr. (1991) J. Immunol. 141, 2783–2789
19. Netea, M. G., Gijzen, K., Coolen, N., Verschueren, I., Figdor, C., Van der Meer, J. W., Torensma, R., and Kullberg, B. J. (2003) Microbes Inf. 5, 985–989
20. Thompson, H. L., and Wilton, J. M. (1992) Clin. Exp. Immunol. 87, 316–321
21. Cambi, A., and Figdor, C. G. (2003) Curr. Opin. Cell Biol. 15, 539–546
22. Netea, M. G., van der Graaf, C., Van der Meer, J. W., and Kullberg, B. J. (2004) J. Leukoc. Biol. 75, 749–755
23. Newman, S. L., and Holly, A. (2001) Infect. Immun. 69, 6813–6822
24. Cambi, A., Gijzen, K., de Vries, J. M., Torensma, R., Joosten, B., Adema, G. J., Netea, M. G., Kullberg, J. B., Romanu, L., and Figdor, C. G. (2003) Eur. J. Immunol. 33, 532–538
25. Netea, M. G., Gow, N. A., Munro, C. A., Bates, S., Collins, C., Ferwerda, G., Hobson, R. P., Bertram, G., Hughes, H. B., Jansen, T., Jacobs, L., Buurman, E. T., Gijzen, K., Williams, D. L., Torensma, R., McKinnon, A., MacCallum, D. M., Odds, F. C., Van der Meer, J. W., Brown, A. J., and Kullberg, B. J. (2006) J. Clin. Investig. 116, 1642–1650
26. Bleijs, D. A., Binnerts, M. E., van Vliet, S. J., Figdor, C. G., and van Kooyk, Y. (2000) J. Cell Sci. 113, 391–400
27. Geijtenbeek, T. B., Torensma, R., van Vliet, S. J., van Duijnhoven, G. C., Adema, G. J., van Kooyk, Y., and Figdor, C. G. (2000) Cell 100, 575–585
28. Kogan, G., Pavlijak, V., and Masler, L. (1988) Carbohydr. Res. 172, 243–253
29. Chovarvaticová, D., Machová, E., Sandula, J., and Kogan, G. (1999) Mutat. Res. 432, 149–156.

FIGURE 5. The mannan epitope is essential for the interaction between C. albicans and DC-SIGN-expressing cells. DC-SIGN-expressing cells bind to C. albicans lacking the O-linked mannan (mnt1/mnt2), to C. albicans lacking the phosphomannose (mmn4) and to C. albicans lacking the terminating β-1,2 mannosese residues (serotype B wild-type as well as Δmmn4), indicating that these moieties do not represent the essential binding epitope. On the contrary, DC-SIGN-expressing cells are unable to bind to C. albicans that has a dramatic reduction (pmr1) or a complete loss (och1) of the branched acid-stable N-linked mannan part. Mannosyl residues not essential for binding are shown in gray.
