Differential High-Affinity Interaction of Dectin-1 with Natural or Synthetic Glucans Is Dependent upon Primary Structure and Is Influenced by Polymer Chain Length and Side-Chain Branching


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ABSTRACT

Glucans are structurally diverse fungal biopolymers that stimulate innate immunity and are fungal pathogen-associated molecular patterns. Dectin-1 is a C-type lectin-like pattern recognition receptor that binds glucans and induces innate immune responses to fungal pathogens. We examined the effect of glucan structure on recognition and binding by murine recombinant Dectin-1 with a library of natural product and synthetic glucans with a pure (1→3)-β-(1→6)-β-glucans as well as nonglucan polymers. Dectin-1 is highly specific for glucans with a pure (1→3)-β-linked backbone structure. Although Dectin-1 is highly specific for (1→3)-β-glucans, it does not recognize all glucans equally. Dectin-1 differentially interacted with (1→3)-β-o-glucans over a very wide range of binding affinities (2.6 mM–2.2 pM). One of the most striking observations that emerged from this study was the remarkable high-affinity interaction of Dectin-1 with certain glucans (2.2 pM). These data also demonstrated that synthetic glucan ligands interact with Dectin-1 and that binding affinity increased in synthetic glucans containing a single glucose side-chain branch. We also observed differential recognition of glucans derived from saprophytes and pathogens. We found that glucan derived from a saprophytic yeast was recognized with higher affinity than glucan derived from the pathogen Candida albicans. Structural analysis demonstrated that glucan backbone chain length and (1→6)-β side-chain branching strongly influenced Dectin-1 binding affinity. These data demonstrate: 1) the specificity of Dectin-1 for glucans; 2) that Dectin-1 differentiates between glucan ligands based on structural determinants; and 3) that Dectin-1 can recognize and interact with both natural product and synthetic glucan ligands.

The innate immune system identifies pathogens based on the carbohydrates, lipids, nucleic acids, and proteins expressed by the microorganism (Akira and Hemmi, 2003). These macromolecular structures are called pathogen-associated molecular patterns (PAMPs). Because they are evolutionarily conserved in microbes and structurally distinct from macromolecules expressed on the surface of mammalian cells, PAMPs are ideal recognition molecules for the innate immune system (Akira and Hemmi, 2003). The innate immune system has evolved receptors capable of recognizing and interacting with pathogens via their PAMPs (Akira and Hemmi, 2003).
Hemmi, 2003; Brown and Gordon, 2003). These evolutionarily conserved receptors are generically classified as pattern recognition receptors (PRRs) (Akira and Hemmi, 2003). The recognition and interaction of PAMPs by PRRs is a critical step in innate immune recognition of diverse pathogens.

Glucans are major structural components of the cell wall of fungi and certain bacteria (Williams et al., 2004). Structurally, glucans are composed of a polymer backbone containing (1→3)-β-D-linked anhydroglucose repeat units (AGRUs) (Fig. 1) (Kapteyn et al., 2000; Klis et al., 2001; Chauhan et al., 2002). Some, but not all, glucan polymers exhibit side-chain AGRUs that branch exclusively from the 6-position of the backbone AGRU (Ensley et al., 1994; Kim et al., 2000; Lowman and Williams, 2001). There is extensive evidence demonstrating that glucans will stimulate innate immunity (Brown and Gordon, 2003; Williams et al., 2004). As a result, glucans have been identified as fungal PAMPs (Brown and Gordon, 2003; Williams et al., 2004).

Brown et al. (2003) have demonstrated that Dectin-1 is the primary PRR for glucans. Dectin-1 is a type II transmembrane receptor containing a single extracellular C-type lectin-like carbohydrate recognition domain and an immunoreceptor tyrosine-based activation motif in the cytoplasmic tail (Brown and Gordon, 2001; Willment et al., 2001; Brown et al., 2002, 2003; Taylor et al., 2002). Dectin-1 is expressed at high levels on blood and splenic monocytes, neutrophils, and alveolar and inflammatory macrophages, and at lower levels by dendritic cells and a subset of T lymphocytes (Willment et al., 2001; Brown et al., 2002; Taylor et al., 2002). Dectin-1 will bind free glucans and zymosan as well as whole Candida albicans and Saccharomyces cerevisiae in a glucan-dependent manner (Brown and Gordon, 2001; Willment et al., 2001; Brown et al., 2002, 2003; Taylor et al., 2002). At this time, there is controversy regarding the precise role of Dectin-1 in fungal infections. Taylor et al. (2007) have reported that Dectin-1 is required for glucan recognition and control of

**Linear**

![Linear Structure](image)

**Branched Structure 1**

![Branched Structure 1](image)

**Branched Structure 2**

![Branched Structure 2](image)

**Fig. 1.** Glucan polymers are composed of glucose monomers that are (1→3)-β-D-linked to form a backbone chain (BC). Glucan polymers can be linear, i.e., nonbranched. The (1→3)-β-D-linked BC polymers can also have (1→6)-β-linked side branches. Side-chain branching can occur at the reducing terminus (RT; Branched Structure 1) or along the polymer backbone (Branched Structure 2). The glucan polymer has a reducing terminus (RT), a nonreducing terminus (NRT), and a backbone polymer chain (BC). The reducing terminus can exist as α or β anomers. SRT is the second glucose subunit from the RT.
C. albicans infection. In contrast, Saigo et al. (2007) have reported that Dectin-1 is required for host defense against Pneumocystis carinii but not against C. albicans. When considered together, these reports indicate that Dectin-1 may be crucial to the innate immune recognition of diverse fungi. Thus, Dectin-1 seems to be an important sentinel receptor for fungal infections (Brown and Gordon, 2003; Williams et al., 2003; Herre et al., 2004b).

Previous reports indicate that the physicochemical properties of glucans (e.g., primary structure, polymer size, surface charge, solution conformation, and side-chain branching) may be important determinants for recognition and interaction with PRRs in the innate immune system (Müller et al., 1996; Mueller et al., 2000). Most studies have used competitive binding experiments with Dectin-1-expressing cell lines, rather than pure receptor, and/or the carbohydrates used were not critically characterized, or they were not pure glucans (Herre et al., 2004a). As a result, the structural features that influence fungal glucan recognition by Dectin-1 have not been elucidated. The purpose of this study was to investigate how glucan polymer structure influences recognition and interaction by murine recombinant Dectin-1.

Materials and Methods

Natural Product Carbohydrate Polymers. A description and comparison of the carbohydrates used in this study are given in Table 1. The heptasaccharide glucan was purchased from the Yazu Suisankagaku Company (Tokyo, Japan). Mannan, a mannotose polymer derived from S. cerevisiae, and laminarin were purchased from Sigma-Aldrich (St. Louis, MO). Glucan phosphate was prepared as described by Williams et al. (1991). Scleroglucan was prepared as described by Pretus et al. (1991). Glucans were isolated from the yeast (bassopore) and hyphal forms of C. albicans as described by Williams et al. (1991), Ensley et al. (1994), and Mueller et al. (2000). Most studies have used competitive binding experiments with Dectin-1-expressing cell lines, rather than pure receptor, and/or the carbohydrates used were not critically characterized, or they were not pure glucans (Herre et al., 2004a). As a result, the structural features that influence fungal glucan recognition by Dectin-1 have not been elucidated. The purpose of this study was to investigate how glucan polymer structure influences recognition and interaction by murine recombinant Dectin-1.

Table 1

| TABLE 1 | Dectin-1 binding constants (IC_{50}) for natural product and synthetic \( \beta \)-D-glucans |
|---|---|---|---|
| Glucan | Type of Glycosidic Linkage | \( M_r \) | IC_{50}^a (\mu M) | CIs (95%) |
| Heptasaccharide | \( \beta-(1\rightarrow3) \) | 1.15 \times 10^3 | >1 mM | |
| Branched heptasaccharide | \( \beta-(1\rightarrow3), (1\rightarrow6) \) | 1.33 \times 10^3 | 0.13 mM | 0.11–0.15 mM |
| Linear octasaccharide | \( \beta-(1\rightarrow3) \) | 1.33 \times 10^3 | 1.1 mM | 1.8–4.3 mM |
| Branched octasaccharide | \( \beta-(1\rightarrow3), (1\rightarrow6) \) | 1.51 \times 10^3 | 1.3 mM | 1.1–1.5 mM |
| Linear nonasaccharide | \( \beta-(1\rightarrow3) \) | 1.51 \times 10^3 | 2.6 mM | 2.0–3.4 mM |
| Branched nonasaccharide | \( \beta-(1\rightarrow3), (1\rightarrow6) \) | 1.69 \times 10^3 | 29 \( \mu \)M | 20–41 \( \mu \)M |
| Linear decasaccharide | \( \beta-(1\rightarrow3) \) | 1.69 \times 10^3 | 0.7 mM | 0.57–0.79 mM |
| Laminarin | \( \beta-(1\rightarrow3), (1\rightarrow6) \) | 7.70 \times 10^3 | 22 mM | 17–29 mM |
| C. albicans hyphal glucan | \( \beta-(1\rightarrow3), (1\rightarrow6) \) | 3.3 \times 10^4 | 440 \( \mu \)M | 350–560 \( \mu \)M |
| Glucan phosphate | \( \beta-(1\rightarrow3) \) | 1.55 \times 10^5 | 2.2 \( \mu \)M | 1.7–2.7 \( \mu \)M |
| Scleroglucan | \( \beta-(1\rightarrow3), (1\rightarrow6) \) | 1.02 \times 10^6 | 102 \( \mu \)M | 83–25 \( \mu \)M |

^a IC_{50} is the carbohydrate concentration required to inhibit 50% of Dectin-1 binding to a glucan-coated biosensor surface (Kougias, et al., 2001).

^b Derived from a natural product source as described by Whelan (1963).

^c Synthetic glucan oligosaccharides were prepared as described by Yu et al. (2005).

^d Obtained from Sigma-Aldrich and described by Kim et al. (2000).

^e As described by Lowman et al. (2003).

^f As described by Williams et al. (1991), Ensley et al. (1994), and Mueller et al. (2000).

^g As described by Pretus et al. (1991).
and solution conformation were established by high-performance GPC/MALLS photometry as reported previously (Williams et al., 1992; Mueller et al., 1995). The samples were dissolved at 3.0 mg/ml and filter-sterilized in 50 mM sodium nitrite mobile phase. The columns were maintained at 37 ± 1°C with continuous mobile phase flow. The system was calibrated using narrow-band pullulan standards. Data were acquired and analyzed using Astra software (version 5.1.2; Wyatt Technology, Santa Barbara, CA).

**Linkage Analysis of Carbohydrates by Gas Chromatography-Mass Spectrometry.** The carbohydrates (1 mg) were dissolved in DMSO (3 ml) and stirred overnight at room temperature. NaOH (5 mg) was added to the solution followed by the addition of 2 ml of CH3I. The solution was stirred for 2 h at room temperature. The methylated material was extracted with CH2Cl2 and was then subjected to hydrolysis with 4 M trifluoroacetic acid at 100°C for 4 h. The alditol was reduced with NaBD4 in water and then acetylated with acetic anhydride using residual sodium acetate as a catalyst. The permethylated alditol acetates were then characterized by gas liquid chromatography-mass spectrometry in the electron impact mode using a DB-17, 30 m capillary column (J&W Scientific, Folsom, CA) as previously described by Monteiro et al. (2000).

**Murine Recombinant Dectin-1.** Murine recombinant Dectin-1 was prepared as described by Brown et al. (2003) and Willment et al. (2001). The protein was expressed as a CD4 fusion protein in human embryonic kidney 293 cells (Willment et al., 2001). We confirmed the identity of the recombinant Dectin-1 by Western blot (Supplemental Fig. 1) analysis, and we used GPC/MALLS to confirm purity and Mr. There was no evidence of multimer formation in the recombinant protein preparation.

**Generation of Glucan-Coated Biosensor Chips.** The diaminopropane (DAP) derivative of glucan phosphate was prepared as described previously (Müller et al., 1996). DAP was attached to the reducing terminus of the carbohydrate by sodium borohydride reduction. As described previously, DAP-glucan phosphate was attached to the surface of a BIAcore CM5 chip (BIAcore, Piscataway, NJ) through the single primary amine placed at the reducing terminus of the glucan molecule (Kougias et al., 2001; Lowe et al., 2001; Rice et al., 2005).

**Receptor Binding Studies.** Binding assays were performed according to Kougias et al. (2001) and Rice et al. (2002). In brief, binding was evaluated at 37 ± 1°C using a Biacore 2000 (BIAcore), a surface plasmon resonance technology that measures mass changes at a biosensor surface. DAP-glucan phosphate was immobilized on a CM5 biosensor surface and used for competition experiments in which various carbohydrates competed for Dectin-1 binding sites. Running buffer contained 150.0 mM NaCl, 10.0 mM HEPES, 3.0 mM EDTA, and 0.005% surfactant P20 (BIAcore). Samples were maintained at 4.0 ± 0.1°C using an Isotemp circulating bath (Fisher Scientific Co., Pittsburgh, PA). Recombinant Dectin-1 was mixed with competing carbohydrates for at least 1 h before injection on the BIAcore instrument. Samples containing a fixed concentration of recombinant Dectin-1 in the absence or presence of competing carbohydrates were alternately injected over the biosensor surface for 10 min, and binding was measured in resonance units (RU) (pg/mm²) equivalent to mass changes at the biosensor surface. After a 20-min dissociation period, the biosensor surface was regenerated with sequential injections at 100 µl/min for 1 min of 0.3% Triton X-100 and guanidine hydrochloride 3.0 M.

**Analysis of Binding Data.** The data were analyzed as previously described by our laboratory (Kougias et al., 2001; Rice et al., 2002). In brief, data were normalized to the baseline established at the start of the experiment and analyzed by unweighted nonlinear regression using Prism 3.0 (GraphPad Software, Inc., San Diego, CA). For competition experiments, the glucans were exposed to fixed concentrations of recombinant Dectin-1 protein in the absence and presence of competitor. RU values for each competitor were further normalized to binding in the absence of competitor (100%) and analyzed using models for competitive displacement at a single binding site. Carbohydrates that compete for the glucan binding site on Dectin decrease its interaction with glucan phosphate on the biosensor surface; binding is similar to control in the presence of carbohydrate concentrations that do not bind to Dectin-1.

**Computational Analysis and Generation of Molecular Models.** Chemical structures were drawn in a linear format (see Fig. 1) using CS Chem-Draw Ultra (version 6.0.1; Cambridge Soft Corporation; Cambridge, MA). The structures were copied into CS Chem3D Ultra. For each polysaccharide, a molecular mechanics minimization was performed using a RMS of 0.005. Next, molecular geometries were generated in the Gaussian Z-matrix style via the CS MOPAC application. For each compound, an Austin-Model (AM1) semiempirical calculation (closed shell, tight convergence criteria) was conducted by using Gaussian (G98W; Gaussian, Inc., Carnegie, PA) (Frisch et al., 2004). *Ab initio* geometry optimizations were subsequently performed using Gaussian 03 at the Hartree-Fock level of theory using the STO-3G (Hrhe et al., 1969; Collins et al., 1976) basis set. Geometry optimizations using implicit solvation were performed with the Onsager method (Onsager, 1936) using the dielectric constant of water (78.39) and the solute radius; calculated with the more accurate molecular volume computation (Volume = Tight). These calculations were performed with a Microway AMD (Microway Technology, Plymouth, MA) dual 64 bit 2.0 GHz central processing unit (CPU) with 4GB RAM running Fedora Core 3 and an Aspen Systems 30 CPU cluster (Wheatridge, CO): each blade containing two 32-bit Xeon 3.2 GHz CPUs with 4GB RAM running Red Hat Linux 8.0.

**Results**

**Murine Dectin-1 Is Highly Specific for Carbohydrate Polymers with a (1–3)-β-d-Linked Anhydroglucose Backbone Structure.** Surface plasmon resonance competition experiments were performed using recombinant Dectin-1 in the absence or presence of varying concentrations of glucan and nonglucan carbohydrate polymers (Table 1). Murine Dectin-1 only recognized and interacted with polymers that contained a (1–3)-β-d-linked glucose backbone. Dectin-1 did not recognize (IC50 > 1 mM) nonglucan carbohydrate polymers, such as pullulan and mannan (Fig. 2). Dectin-1 did not interact with a plant-derived (barley) glucan, which has a mixed-linkage (1→3)/(1→4)-β backbone structure (data not shown). Furthermore, Dectin-1 did not interact with a glucan that was exclusively composed of a (1→6)-β-linked glucose backbone (Fig. 2). In addition, Dectin-1 did not interact with linear (1→3)-β-d-glucan oligosaccharides composed of ≤7 glucose subunits (Table 1) (Lowe et al., 2001). The minimal binding subunit for recombinant Dectin-1 is a (1→3)-β-d-glucan oligosaccharide containing a backbone with seven glucose subunits and a single (1→6)-β-linked side-chain branch at the nonreducing terminus. This indicates that Dectin-1 is highly specific for (1→3)-β-d-glucans and that the minimal binding subunit for murine recombinant Dectin-1 is a branched heptasaccharide glucan.

**Differential Recognition of (1–3)-β-Glucans by Murine Dectin-1.** For each glucan studied, the binding interaction was specific, dose-dependent, competitive, and indicative of a single binding site (Fig. 3). However, we found that Dectin-1 did not interact with all glucans equally (Table 1 and Fig. 3). Indeed, Dectin-1 differentially interacted with (1→3)-β-d-glucans over a very wide range of binding affinities (IC50 = 2.6 mM–2.2 pM). The lowest binding affinity was IC50 = 2.6 mM for a nonbranched octasaccharide glucan. The highest affinity interaction was glucan phosphate at IC50 =
Differential Recognition of Synthetic Glucan Oligosaccharides by Dectin-1. Glucans derived from natural sources usually contain a complex mixture of structures, molecular weights, and solution conformations (Pretus et al., 1991; Mueller et al., 1995; Kim et al., 2000). To critically evaluate the structure/activity relationships of glucan and Dectin-1, it would be preferable to have glucans with highly specific structures. To achieve this goal, we synthesized a small library of linear and branched (1→3)-β-D-glucan oligosaccharides according to the method of Yu et al. (2005) (Fig. 5). We synthesized glucan oligosaccharides consisting of 8, 9, and 10 glucose subunits. The synthetic glucan oligosaccharides were linear or had a single glucose subunit as a side-chain branch (Yu et al., 2005). Dectin-1 recognized and interacted with all of the synthetic glucans, albeit at lower affinity than the higher Mr natural product glucans (Table 1).

However, binding affinity for the synthetic glucans did increase as the number of glucose subunits in the polymer backbone increased. In general, the branched glucan oligosaccharides were recognized by Dectin-1 with higher affinity than the comparable linear glucan. By way of example, the highest affinity binding observed for the synthetic glucan polymers was the branched nonasaccharide (IC50 = 29 μM) (Fig. 5). The branched nonasaccharide has a backbone containing nine glucose subunits and a single glucose side-chain branch for a total of 10 glucose subunits. There was a 1000-fold increase in binding affinity between the linear and branched nonasaccharide (2.6 mM versus 2.9 μM). There was a ~270-fold increase in binding affinity between the branched nonasaccharide and the linear decasaccharide (0.7 mM versus 2.9 μM). This demonstrates that Dectin-1 recognizes and interacts with synthetic glucan ligands, that the binding affinity increases with polymer length, and, more significantly, the presence of even a single (1→6)-β-linked glucose side-chain branch can dramatically increase the recognition of glucan by murine recombinant Dectin-1 (Fig. 5).

Computational Analysis and Molecular Modeling of Linear and Branched Synthetic Decasaccharide Glucans. The data presented in Fig. 5 suggest that a more in-depth understanding of the structure of the synthetic decasaccharide glucans might provide insights into how Dectin-1 recognizes its ligand. Therefore, theoretical optimized linear molecular geometries, relative energies (kilocalorie/mole), and molar volumes for the synthesized decasaccharide and branched nonasaccharide were computationally investigated (Fig. 6). As expected, the molecular models reveal a helical backbone structure in both the linear and branched synthetic decasaccharides. The most notable differences between the two structures are the overall size of the polymer and the single glucose side-chain branch extending away from the polymer backbone (Fig. 6).

Discussion

In this study, we demonstrate that recombinant murine Dectin-1 is highly specific for glucans that have a (1→3)-β-
D-glucopyranosyl backbone. Dectin-1 did not recognize non-β-linked carbohydrate polymers (e.g., mannan or pullulan), and Dectin-1 did not interact with plant-derived glucans (e.g., barley glucan) that have a mixed-linkage polymer backbone characterized by alternating regions of (1→3)-β and (1→4)-β linkages (Aman and Graham, 1987). The specificity of the interaction was further demonstrated by the observation that a (1→6)-β-linked glucan is not recognized by Dectin-1.

Although Dectin-1 is highly specific for (1→3)-β-D-glucans, it does not recognize all (1→3)-β-D-glucans equally. Dectin-1 differentially interacted with (1→3)-β-D-glucans over a very wide range of binding affinities (2.6 mM-2.2 pM). Indeed, one of the most striking observations that emerged from this study was the differential recognition of glucans derived from saprophytes and pathogens. We found that glucan phosphate, which is derived from a saprophytic yeast, was recognized with higher affinity than either blastospore or hyphal glucan derived from the pathogen *C. albicans*. We have compared and contrasted the structure of *C. albicans* and *S. cerevisiae* glucans used in this study (Lowman et al., 2003). We found that “the level of (1→6) side chain branching” is higher in *C. albicans* glucan than in *S. cerevisiae* glucan (Lowman et al., 2003). These data suggest that the presence, degree, and perhaps length of side-chain branches can negatively and positively influence Dectin-1 recognition of glucans. We also found that binding affinity tended to increase as polymer size, and by extension *Mw*, increased. Interestingly, the observation that greater molecular size increased the affinity of Dectin-1 for glucans did not apply to all of the glucans tested. Scleroglucan was the largest polymer tested, but it interacted with lower affinity than glucan phosphate. This observation is consistent with our previous report using a human monocyte cell line assay (Mueller et al., 2000). It was initially thought that differences in solution conformation might account for the difference in binding affinity; however, our results suggest that this is not the case. Scleroglucan and glucan phosphates were characterized by gas chromatography/mass spectrometry-linkage analysis. Glucan phosphate is composed of 3-substituted glucose. In contrast, scleroglucan contained portions of 4-substituted glucose residues, indicating that scleroglucan has backbone regions that are not (1→3)-β-linked. Because our data indicate that Dectin-1 does not interact with mixed-linkage polymers, we conclude that scleroglucan has sufficient long regions of (1→3)-β-linked backbone to facilitate Dectin-1 recognition. As a result, scleroglucan is recognized by Dectin-1, but the presence of 4-substituted glucose would cause our data indicate that Dectin-1 does not interact with glucan phosphate. n = 4/group/concentration.

**Fig. 3.** Differential recognition and binding of natural product glucans by recombinant murine Dectin-1. Dectin-1 bound laminarin, glucan phosphate, and scleroglucan with varying affinities. The competition of each carbohydrate completely inhibited the interactions with high affinity and characteristics of a single binding site. Glucan phosphate interacted with Dectin-1 [2.2 pM; 95% confidence interval (CI) of 1.7–2.7 pM]. Scleroglucan (102 pM; 95% CI of 83–125 pM) and laminarin (22 nM; 95% CI of 17–29 nM) also interacted with Dectin-1 but at lower affinity than glucan phosphate. n = 4/group/concentration.

**Fig. 4.** Differential binding of glucans isolated from saprophytic versus pathogenic fungi. Glucan derived from *S. cerevisiae* was recognized with the highest affinity, followed by *C. albicans* hyphal glucan and *C. albicans* blastospore glucan. All of the glucans were isolated and solubilized as described by Lowman et al. (2003) and Williams et al. (1991). n = 4/group/concentration.

**TABLE 2**
Comparison of the relative ratios of monosaccharide linkage types present in glucan phosphate, scleroglucan, and laminarin

<table>
<thead>
<tr>
<th>Monosaccharide/Linkage</th>
<th>Glucan Phosphate</th>
<th>Scleroglucan</th>
<th>Laminarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc-(1-3)</td>
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<td>40</td>
<td>7</td>
</tr>
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<td>Glc-(1-3)</td>
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<td>36</td>
<td>40</td>
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<td>2</td>
<td>16</td>
<td>Trace</td>
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<td>1</td>
</tr>
<tr>
<td>Glc-(1-3)</td>
<td>8</td>
<td>18</td>
<td>4</td>
</tr>
</tbody>
</table>

*Indicates that trace amounts were detected, but they were below the limit of quantification.

The data in Table 2 and Fig. 4 suggest that the affinity of Dectin-1 for glucans is affected by the presence and degree of side-chain branching.
residues in the backbone may limit interaction of multiple Dectin-1 molecules with the glucan and, thus, may account for the lower affinity. Another possible explanation is that the mixed-linkage backbone structure results in solution structures that are not optimal for Dectin-1 interaction. In any case, these data strengthen the conclusion that Dectin-1 is highly specific for glucose polymers with a (1→3)-β-linked backbone.

We also determined that Dectin-1 recognition of glucan ligands requires a backbone chain length of at least seven glucose subunits and at least one glucose side-chain branch. Thus, our data support and extend the conclusion (see Brown and Gordon, 2001) that the minimal binding unit for Dectin-1 is highly specific for glucose polymers with a (1→3)-β-linked backbone.

Previous data indicate that the presence of (1→6)-β-linked side-chain branches increases the affinity of pattern recognition receptors for glucan (Mueller et al., 2000). However, because of the inherent difficulties in completely deciphering the fine structure of a glucan polymer isolated from a natural source, it has been exceedingly difficult to definitively confirm or refute this conclusion. This is particularly true with regard to the precise location and length of side-chain
branches. To address this issue, we synthesized linear (non-branched) and branched (1→3)-β-D-linked glucan oligosaccharides (i.e., synthetic glucans with a single (1→6)-β-D-linked side-chain branch at the second glucose residue from the nonreducing terminus) (Yu et al., 2005). These synthetic glucans provided a unique opportunity to investigate the interaction of Dectin-1 with glucans whose fine structure is known. The synthetic glucans were composed of eight, nine, or 10 glucose residues because a linear heptasaccharide glucan is not recognized by Dectin-1. Each synthetic glucan was bound by Dectin-1, clearly demonstrating that Dectin-1 is capable of interacting with synthetic glucan ligands. To the best of our knowledge, this is the first report demonstrating that purely synthetic glucans are recognized by murine Dectin-1. The most striking observation was that binding affinity increased in synthetic glucans containing a single glucose side-chain branch. The increase in the affinity of the branched nonasaccharide versus the other synthetic glucans suggests the importance of several pivotal modifications brought together in this structure. The marked increase in affinity that accompanies a single side-chain branch supports the importance of side-chain branching, a key structural feature that differentiates glucans from various sources. Adachi et al. (2004) have identified Trp221 and His223 as critical for the interaction of β-glucans with Dectin-1. In a recent study, Brown et al. (2007) have elucidated the crystal structure of Dectin-1. Their model revealed a shallow groove running between Trp221 and His223 (Brown et al., 2007). Our results suggest that (1→3)-β-D-glucans with a polymer backbone composed of seven or more glucose repeat subunits can interact with the Trp221/His223 groove in Dectin-1. Molecular modeling of the synthetic glucans indicates that six glucose subunits are required for one helical turn of the glucan polymer (Fig. 6). Thus, a branched glucan polymer containing at least eight glucose subunits (branched heptasaccharide) and slightly more than one full helical turn is required for interaction with Dectin-1. In addition, the presence of a single (1→6)-β-D-linked side-chain branch on a (1→3)-β-D-glucan polymer can dramatically increase recognition by Dectin-1. To better understand how glucan structure may influence Dectin-1 binding, theoretical optimized molecular geometries (linear conformations) for the synthetic linear and branched glucans were computationally investigated. The data reveal differences between the linear decasaccharide and the branched nonasaccharide, both of which contain 10 glucose residues. The most obvious difference is the presence of the side-chain branch. Comparing the solvated linear conformations revealed that the linear decasaccharide was thermodynamically more stable (4.81 kcal/mol) than the branched nonasaccharide. Furthermore, the molar volumes were different in that the linear decasaccharide (962 cm$^3$/mol) was larger than the branched nonasaccharide (854 cm$^3$/mol). The modeling data also provided insights into how Dectin-1 may interact with glucans. The (1→3)-β backbone linkages cause the polymer to assume a helical conformation (Fig. 6). We speculate that the helical nature of glucan polymers may facilitate the interaction of glucans with the Trp221/His223 groove in Dectin-1 (Adachi et al., 2004). Thus, the Dectin-1 binding pocket may be a groove that accommodates the helical glucan structure. The increased affinity of Dectin-1 for glucans with side-chain branches suggests that the binding pocket may have a unique structure that preferentially interacts with the branched glucan. As noted above, the greatest affinity interaction was observed for high $M_t$ glucans that contain hundreds of glucose subunits, such as glucan phosphate. Using the synthetic glucan binding data, we calculated that each glucose subunit in the backbone structure contributes ~200 calories of energy to the binding interaction (data not shown). We speculate that large glucan polymers are bound by many Dectin-1 molecules along the repeating subunit polymer backbone and, as a result, increase affinity in an additive fashion. It is also possible that Dectin-1 forms multimers on the cell membrane, thus facilitating high-affinity interaction. However, we were not able to detect multimer formation in our Dectin-1 preparation. This does not preclude multimer formation on cell membranes, it merely indicates that our Dectin-1 preparation did not form multimers under the conditions used and that multimer formation is not required for high-affinity interaction of Dectin-1 with glucan ligands.

In conclusion, we observed that Dectin-1 is highly specific for glucans that have a (1→3)-β-D-glucopyranosyl backbone. However, Dectin-1 also differentially recognizes glucans based on structural factors that include polymer length and side-chain branching. These observations provide new insight into how a sentinel pattern recognition receptor in the innate immune system recognizes and interacts with fungal pathogen-associated molecular patterns.

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Structure Activity Relationships of Glucan and Dectin-1

References


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