

A (1→3)- β -D-linked heptasaccharide is the unit ligand for glucan pattern recognition receptors on human monocytes

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ABSTRACT – Glucans are fungal cell wall polysaccharides which stimulate innate immune responses. We determined the minimum unit ligand that would bind to glucan receptors on human U937 cells using laminarin-derived pentaose, hexaose, and heptaose glucan polymers. When U937 membranes were pretreated with the oligosaccharides and passed over a glucan surface, only the heptasaccharide inhibited the interaction of glucan with membrane receptors at a K_d of 31 μ M (95% CI 20–48 μ M) and 100% inhibition. However, the glucan heptasaccharide did not stimulate U937 monocyte NF κ B signaling, nor did it increase survival in a murine model of polymicrobial sepsis. Laminarin, a larger and more complex glucan polymer ($M_w = 7\,700$ g/mol), only partially inhibited binding (61 \pm 4%) at a K_d of 2.6 μ M (99% CI 1.7–4.2 μ M) with characteristics of a single binding site. These results indicate that a heptasaccharide is the smallest unit ligand recognized by macrophage glucan receptors. The data also indicate the presence of at least two glucan-binding sites on U937 cells and that the binding sites on human monocyte/macrophages can discriminate between glucan polymers. The heptasaccharide and laminarin were receptor antagonists, but they were not receptor agonists with respect to activation of NF κ B-dependent signaling pathways or protection against experimental sepsis.
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glucan / receptors / heptasaccharide / NF κ B / macrophage / monocyte / sepsis

1. Introduction

The innate immune system has evolved a complex network of receptors which rapidly identify pathogens based on the carbohydrates, lipids and proteins expressed by the organism [1–3]. Glucans are (1→3)- β -D-linked polymers of glucose that are part of the outer cell wall of saprophytic and pathogenic fungi [4, 5]. They are also released from the fungal cell wall as exopolymers [6]. Recent data indicate that glucans are released from the

cell wall of pathogenic fungi into the peripheral circulation of patients with systemic or deep fungal infections [7, 8]. In vivo administration of chemically pure glucans will activate a wide array of innate host defenses [9, 10]. This is due, in part, to the ability of these carbohydrates to interact with membrane receptors and stimulate pro-inflammatory and immunoregulatory signaling pathways (NF κ B and NF-IL6) in leukocytes [3, 11–13]. Glucans are structurally distinct from the carbohydrates expressed on the surface of mammalian cells, thus they are ideal recognition molecules [1–3]. It has been postulated that cell wall glucans are fungal pattern recognition molecules for the innate immune system [1–3].

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Many species have pattern recognition receptors or proteins for (1→3)- β -D-glucans [3, 14–15]. Ligation of the receptor(s) with glucan stimulates innate immunity [3, 14–15]. In mammals, glucans induce biological activity through interaction with receptors on macrophages, neutrophils and NK cells [3, 16–17]. Binding of (1→3)- β -D-glucan in human and murine monocytes/macrophages is specific, saturable and susceptible to displacement by other (1→3)- β -D-glucans [3, 18]. There are multiple glucan receptors on human monocytes [3]. The receptors can distinguish between (1→3)- β -D-glucan polymers based on structure and solution conformation [3], and there are large affinity differences (24 μ M to 11 nM) between glucan polymers derived from various sources [3]. We have observed that certain glucans appear to interact non-selectively with glucan-binding sites, while other glucans preferentially interact with only one site [3]. The physical and/or functional basis for this selectivity among glucan receptors is not clear. However, it may explain, in part, the observation that glucans derived from different sources and/or by different extraction methods can exert varied effects on immune function [11–12, 19]. At present, we do not know the minimum physicochemical requirements (molecular weight, branching, solution conformation, etc.) for glucan polymer interaction or expression of immunobiological activity.

Janusz et al. reported that a (1→3)- β -linked yeast heptaglycoside (degree of polymerization (Dp) = seven glucose subunit) inhibited monocyte phagocytosis of zymosan particles [20]. They concluded that this seven-subunit glucan was a 'unit ligand for human monocyte β -glucan receptors' [20]. While this was an intriguing observation, the results of Janusz et al. [20] were equivocal because they did not characterize the heptaglycoside and, therefore, could not confirm that the carbohydrate they employed was a (1→3)- β -D-linked glucan. They did not determine whether the polymer was linear or branched, nor did they employ oligosaccharides with Dp other than seven to confirm that the heptaglycoside was the minimum binding unit [20]. In addition, Janusz et al. employed a phagocytosis inhibition assay which did not directly assess ligand receptor interactions, nor did they evaluate the immunobiological properties of the heptaglycoside [20]. Consequently, a number of important questions remain to be answered.

Recent advances in natural product carbohydrate chemistry and characterization have made it possible to obtain linear, non-branched (1→3)- β -D-linked glucan oligosaccharides that are five, six or seven glucose subunits in length [21]. We evaluated the interaction of glucan oligosaccharides with human monocyte U937 membranes. Glucans have been reported to stimulate macrophage and neutrophil NF κ B signaling pathways [3, 11–13] and increase resistance to experimental infectious diseases [22]. In an attempt to correlate ligand receptor interaction with expression of biological activity the oligosaccharides were evaluated for their ability to stimulate monocyte NF κ B nuclear binding activity and modulate survival in a murine model of polymicrobial sepsis.

2. Materials and methods

2.1. Carbohydrate polymers

Chemically pure laminaripentaose, laminarihexaose and laminariheptaose isolated from hydrolysates of pachyman from *Poria cocos* were obtained from Seikagaku (Tokyo, Japan). Laminarin was obtained from Sigma (St Louis, MO) and characterized according to previously published methods [23]. Laminarin is a (1→3)- β -D-linked glucan polymer with (1→6)- β -linked side chain branches occurring at a frequency of one branch point every ten glucose subunits along the polymer backbone [18, 23]. It has a polydispersity of 1.17 [18, 23]. Glucan phosphate was prepared and quality controlled in our laboratory [4, 23–25]. All of the polymers were screened for endotoxin contamination with the Endospecy assay (Seikagaku/Cape Cod Associates, Falmouth, MA) according to the manufacturer's instructions.

2.2. 13 C-nuclear magnetic resonance analysis of oligosaccharides

The primary structure of the carbohydrate polymers were confirmed by variable temperature Fourier transformed nuclear magnetic resonance (FT- 13 C-NMR) in DMSO- d_6 at a concentration of 50 mg/mL as previously described [4].

2.3. Diaminopropane derivatized glucan phosphate

The diaminopropane (DAP) derivatives of glucan phosphate were prepared by Dr Harry Ensley, Department of Chemistry, Tulane University. DAP was attached to the reducing terminus of the carbohydrate polymer by sodium borohydride reduction [18]. The reaction mixture was dialyzed against 18-MO Ω m ultrapure pyrogen-free water and the glucan was stored as a sterile filtered liquid at 4 °C. Aliquots of the DAP-glucan were analyzed by aqueous gel permeation chromatography/multi-angle laser light scattering (GPC/MALLS) [25] and 13 C-NMR [4] to confirm that the molecular weight, polydispersity, primary structure and solution conformation were not altered by the derivatization.

2.4. Mice

Age- and weight-matched male ICR/HSD mice were obtained from Harlan Sprague Dawley (Indianapolis, IN). The mice were maintained on standard lab chow and water ad libitum with a 12-h light–dark cycle. Serologic testing confirmed that the mice were virus free. All animal procedures were reviewed and approved by the institutional review board for animal care at the James H. Quillen College of Medicine, East Tennessee State University.

2.5. Human monocyte cell line

The human promonocytic cell line, U937, was maintained in our laboratory in RPMI-1640 (Mediatech, Washington DC) containing 9% heat-inactivated calf serum, 1% heat-inactivated fetal bovine serum (HyClone corp., Logan, UT), and 0.2% (v/v) penicillin-streptomycin (Sigma Chemical Co., St Louis, MO). U937 cells were grown as a suspension culture at 37 °C and 5% CO $_2$ tension in a humidified environment and harvested during logarithmic growth.

2.6. Membrane isolation

Cells were centrifuged at 1 200 *g* for 10 min, resuspended in 12 mL of phosphate-buffered saline (PBS) and counted in a hemocytometer. The cells were centrifuged at 1 200 *g* for 10 min and resuspended in PBS containing protease inhibitor cocktail (Sigma Chemical Co, St. Louis, MO) at 10 $\mu\text{L}/10^6$ cells. The mixture was placed on ice and sonicated three times at 35% power for 30 s. Following centrifugation at 650 *g* for 10 min at 4 °C to spin out nuclei, the supernatant was centrifuged in a Beckman 80 Ti rotor at 68 000 rpm (435 000 *g*) for 30 min at 4 °C. The pellet containing membranes was resuspended in 2 mL of Hank's balanced salt solution (Mediatech, Herndon, VA). Aliquots of the membranes were analyzed for protein content using the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL) and stored in liquid nitrogen for later use.

2.7. Generation of glucan-coated BIA sensor chips

The BIAcore CM5 surface consists of an extended carboxymethyl dextran hydrogel that allows immobilization of a ligand using conventional carbodiimide coupling chemistry [18, 26]. DAP glucan was attached to the carboxymethyl surface of the BIAcore CM5 chip through the single primary amine placed at the reducing terminus of the glucan molecule. The CM5 surface was activated by exposing it to 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 25 mM *N*-hydroxysuccinimide for 7 min at a flow rate of 5 $\mu\text{L}/\text{min}$. The functional groups on the CM5 surface remain active for only a short period of time. DAP glucan was immediately injected over the surface at 5 $\mu\text{L}/\text{min}$ for 7 min; this injection typically resulted in the immobilization of 100–500 pg/mm^2 . Because DAP glucan reacts slowly, the activation and immobilization cycle was repeated as necessary to achieve the desired density of DAP glucan at the CM5 surface. The fourth flowcell served as a control for bulk shift and non-specific binding to dextran: it was not exposed to DAP glucan. After the desired amount of DAP glucan was immobilized, all flow cells were exposed to 1 M ethanolamine hydrochloride (pH 8.5) at 5 $\mu\text{L}/\text{min}$ for 7 min to deactivate any remaining carboxysuccinamide groups on the dextran surface.

2.8. Binding experiments using immobilized glucan

Experiments using the CM5 sensor chip with immobilized DAP-glucan phosphate were performed at 37 °C with a pH 7.4 buffer containing 150 mM NaCl, 10 mM HEPES, 3 mM EDTA and 0.005% P20 surfactant (BIAcore, Piscataway, NJ). In saturation experiments, membranes prepared from U937 cells were randomly applied at concentrations of 0.1–100 $\mu\text{g}/\text{mL}$ in HEPES buffer in plastic or siliconized glass vials. A continuous flow of 25 $\mu\text{L}/\text{min}$ buffer was initiated to establish a baseline for measurement of resonance units (RUs). Following KINJECT exposure for 3 min and dissociation for 3 min, the surface was regenerated by consecutive 1-min exposures to Triton X-100 (0.3%) and guanidine hydrochloride (3 M) at 100 $\mu\text{L}/\text{min}$.

2.9. Competition studies

For competition experiments, samples containing a fixed concentration of U937 membranes (1 $\mu\text{g}/\text{mL}$) in the absence and presence of competitor were alternately injected. Membranes were applied using KINJECT for 3 min followed by continuous buffer flow for 3 min to allow partial dissociation of membranes from the carbohydrate surface. The surface was regenerated by consecutive 1-min exposures to Triton X-100 (0.3%) and guanidine hydrochloride (3 M) at 100 $\mu\text{L}/\text{min}$.

2.10. Data analysis for the binding studies

In competitive binding experiments, the DAP-glucan phosphate surface was exposed to membrane protein in the absence and presence of oligosaccharides. Data were normalized to the baseline established at the start of the experiment and responses occurring in a control flowcell lacking DAP-glucan phosphate were subtracted from experimental values. We found that the bulk shift occurs over approximately 15 s, so we estimated the amount of membrane protein bound to the surface by measuring the increase in RUs at 20–30 s after changing from membrane to buffer exposure. Inhibition was normalized to the RU values in the absence of competing oligosaccharide and fit by unweighted non-linear regression using GraphPAD Inplot (San Diego, CA) applying a one-site competition model with fixed minimum and maximum inhibition as appropriate. Values are expressed as mean \pm SEM.

2.11. NF κ B assay

NF κ B binding activity was assessed by the electrophoretic mobility shift assay [27, 28]. Five micrograms of protein was incubated with 1 mL ^{32}P -labeled NF κ B oligonucleotide (17.5 fmol) and 2 μL Gel Shift Binding Buffer (Promega, Madison, WI) for 20 min at room temperature. Loading buffer (5 μL) was added and the samples were run on 16 \times 18 cm non-denaturing 5% acrylamide gels at 90 V until dye fronts moved off the gels (approx. 2–3 h). The gels were dried, exposed to X-ray film and the autoradiograms were quantified by scanning densitometry. Oligonucleotides bearing the NF κ B consensus binding site were obtained from Promega. The sequence of the ds-oligo is as follows: 5'-AGT TGA GGG GAC TTT CCC AGG C-3'.

2.12. Protocol for NF κ B study

U937 cells were plated (1 \times 10⁶/well) overnight prior to addition of glucan oligosaccharides (1 $\mu\text{g}/\text{mL}$), or LPS (1 $\mu\text{g}/\text{mL}$). RPMI-1640 media served as the negative control. The cells were incubated for 90 min, the nuclear protein was harvested and NF κ B binding activity assessed.

2.13. Cecal ligation and puncture study

Cecal ligation and puncture (CLP) was performed according to the method of Williams et al. [29] as described by Baker [30], Ayala [31] and colleagues. CLP mice were randomized into two groups of ten mice per group. Laminariheptaose glucan was administered intraperitoneally (i.p.) (40 mg/kg) 1 h prior to or 15 min after induction of CLP to one group. The control group received isovolumetric dextrose and water. As a positive control, other groups

of mice were treated i.p. (40 mg/kg) with glucan phosphate 1 h prior to induction of CLP [29]. The mice were followed for survival for 13 days.

2.14. Data analysis for transcription factor and survival studies

Group means and time point means within each group were compared with the least significant difference procedure (if the ANOVA *F*-test is significant) or Tukey's procedure (if ANOVA is not significant). Differences in survival between the study groups were tested with the log-rank procedure and Cox's proportional hazard procedure. Probability levels of 0.05 or smaller were used to indicate statistical significance.

3. Results

3.1. ^{13}C -NMR analysis of heptaose oligosaccharide

Figure 1 shows a comparison of the ^{13}C -NMR spectra for the heptaose glucan employed in the competition studies and a linear (1 \rightarrow 3)- β -D-glucan standard derived from *Saccharomyces cerevisiae* [4]. Carbon assignments are given above each of the major glucose peaks. The carbon signals from the 3rd, 4th, 5th and 6th glucose units (numbered from the reducing end) of the heptaose correspond well with the signals observed for standard glucan. The smaller peaks observed in the heptaose spectrum have been assigned to the carbons of 1st, 2nd and 7th glucose units of the heptaose. The small signal at 103.5 ppm is assigned to the C-1 of the 7th glucose unit, and the peaks at 96.1 and 91.5 ppm are assigned to the β -C-1 and α -C-1 of the reducing terminus, respectively. These data correspond well with the report of Kim et al. [21]. The corresponding signals for reducing termini are not observed in the glucan standard spectrum due to its much larger native molecular weight (1.06×10^5 g/mol) [32]. The set of narrow line-width peaks corresponds to the reducing termini of the heptaose oligosaccharide [21].

3.2. Competition with pentaose, hexaose and heptaose

Competition experiments were performed using 1 $\mu\text{g}/\text{mL}$ U937 membrane protein in the absence or presence of pentaose, hexaose or heptaose. There was no binding inhibition in the presence of up to 100 μM pentaose (figure 2) or 100 μM hexaose (data not shown) ($n \geq 6$). Competition with heptaose was evident at concentrations between 10 μM and 100 μM (figure 3). Inhibition was concentration dependent (IC_{50}) and binding was completely inhibited at 10^{-3} M (figure 4). The competition curve was consistent with recognition of a single-affinity binding site with a K_d of 31 μM (95% CI 20–48 μM) (figure 4). For comparison, the competition curve for glucan phosphate was included. Inhibition was concentration dependent and completely inhibited binding (figure 4) with characteristics of a single binding site. The K_d for glucan phosphate was 5.2 μM (95% CI 3.8–7.2 μM). The K_d for glucan phosphate was significantly lower ($P < 0.05$) than the K_d for heptaose.

3.3. Competition with laminarin

Laminarin also effectively inhibited binding of U937 membranes to a glucan-coated BIA sensor surface (figure

Glucan standard

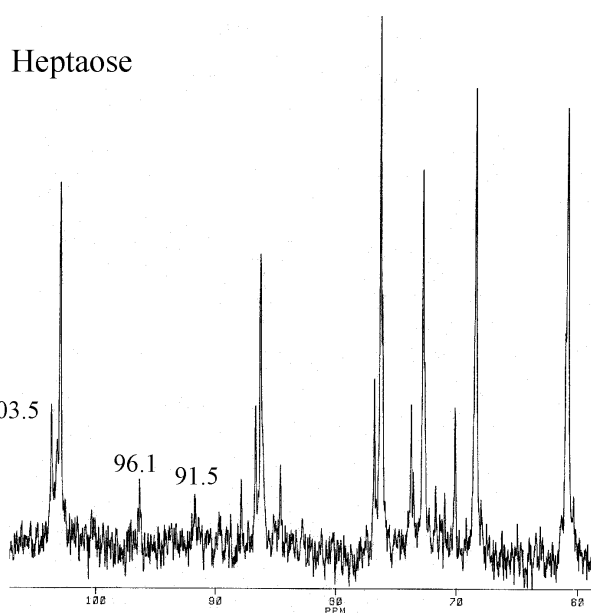
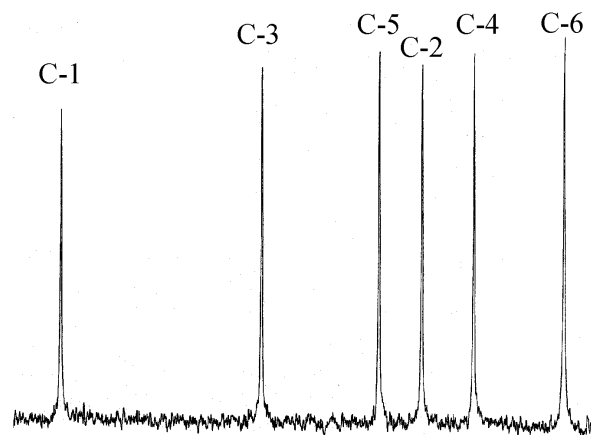


Figure 1. ^{13}C -NMR spectrum of the heptaose oligosaccharide. The six major glucose peaks are labeled C-1 through C-6. The spectrum is consistent with the (1 \rightarrow 3)- β -D-glucan assignment. The set of narrow line-width peaks are the reducing termini of the polymer strand. For NMR analysis the heptaose glucan was dissolved in deuterated dimethyl sulfoxide at 50 mg/mL.

5). Competition with laminarin was concentration dependent and consistent with recognition of a single-affinity binding site with a K_d of 2.6 μM (95% CI 1.7–4.2 μM). In contrast to heptaose, laminarin inhibited only $61 \pm 4\%$ of total binding (figure 5). Approximately 39% of total binding could not be inhibited by competition with laminarin, even at high concentrations of inhibitor.

3.4. Glucan oligosaccharides or laminarin did not stimulate NF κ B binding activity in a human monocyte-like cell line

None of the glucan oligosaccharides (pentaose, hexaose or heptaose) stimulated NF κ B nuclear binding activity

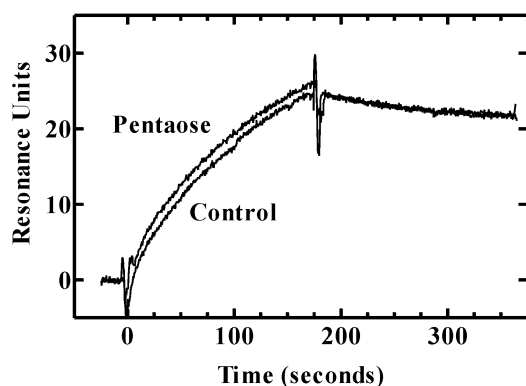


Figure 2. The pentaose polymer did not inhibit binding of U937 membranes to a DAP-glucan phosphate surface at concentrations up to 100 μM . Binding at the sensor surface is shown in resonance units (pg/mm^2). Similar results were obtained for the hexaose polymer.

when incubated with the human monocyte-like cell line, U937, for 90 min (figure 6). Laminarin also failed to stimulate NF κ B nuclear binding activity (data not shown). However, as previously reported, LPS (1 $\mu\text{g}/\text{mL}$) [12] was a potent stimulus for NF κ B nuclear binding activity in this cell line (figure 6).

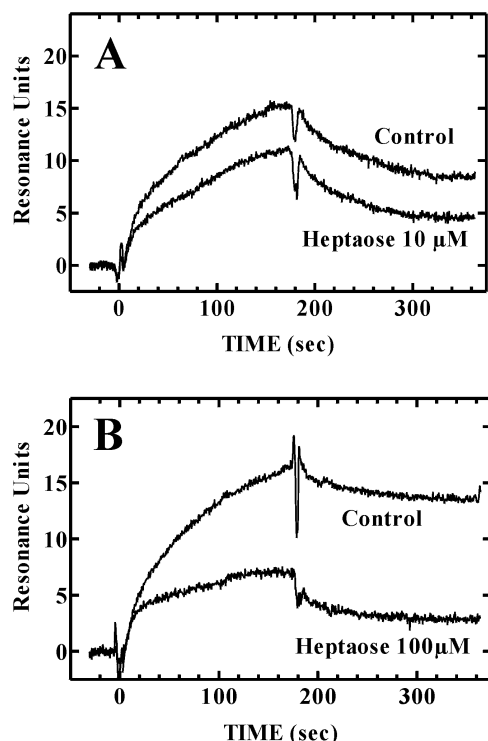


Figure 3. Sensorgrams showing the binding of U937 membranes to a DAP-glucan phosphate surface in the absence (control) and presence of 10 μM (A) and 100 μM (B) heptaose. Heptaose inhibition of U937 membrane binding was concentration dependent.

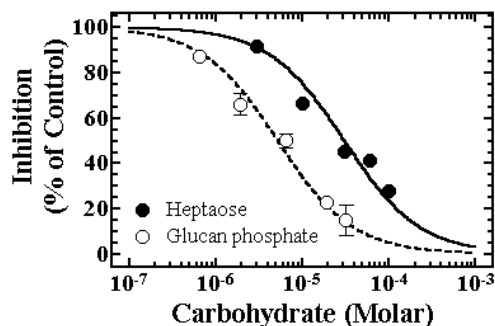


Figure 4. The inhibition of U937 membrane binding by heptaose was concentration dependent and fit a model for inhibition at a single binding site (closed circle). Complete inhibition of binding was observed at competitor concentrations of $\sim 10^{-3}$ M. The K_d of heptaose was 31 μM . The inhibition of U937 membrane binding by glucan phosphate is also shown for comparison (open circle). The K_d for glucan phosphate was 5.2 μM .

3.5. Pre- or post-treatment with heptaose polymers does not increase resistance of ICR/HSD mice to polymicrobial sepsis

We have shown that (1 \rightarrow 3)- β -D-glucans with M_w of $\geq 1.4 \times 10^4$ g/mol will increase resistance to experimental peritonitis [29, 33, 34]. In the present study, we observed that the heptaose polymer did not increase median survival time or long-term survival in the cecal ligation and puncture model when compared to the dextrose control (figure 7). Glucan phosphate increased long-term survival, as denoted by a 60% ($P < 0.05$) long-term survival (figure 7). The effect of glucan phosphate in the CLP model is consistent with previous reports from our laboratory [29]. It is important to note that the heptaose glucan has an M_w of 1 153 g/mol, while the glucan phosphate has an M_w of 1.25×10^5 g/mol [25].

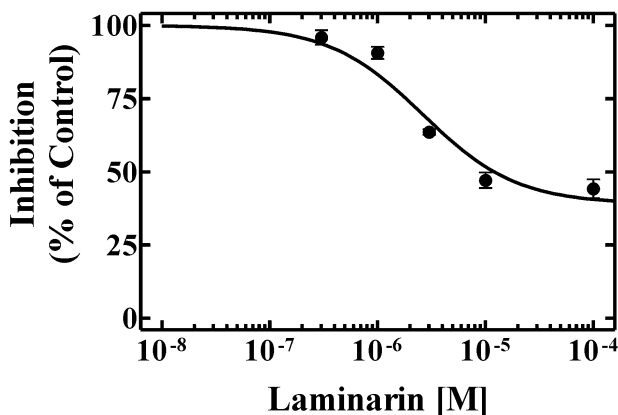


Figure 5. The inhibition of U937 membrane binding by laminarin was concentration dependent and fit a model for inhibition at one of two binding sites. The K_d of heptaose was 2.6 μM . However, only $61 \pm 4\%$ of binding could be inhibited over a dose range of 10^{-8} to 10^{-4} M. Approximately 39% of the U937 membrane binding could not be inhibited by laminarin.

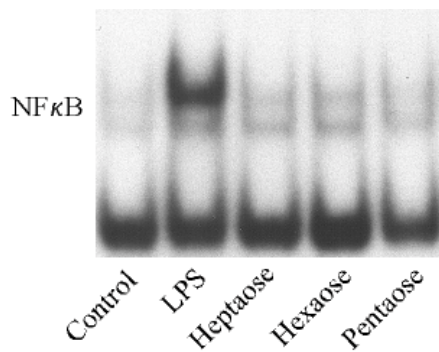


Figure 6. None of the (1→3)- β -D-linked oligosaccharides stimulated macrophage NF κ B nuclear binding activity. U937 cells were incubated with oligosaccharides (1 μ g/mL) for 90 min prior to harvesting nuclear protein. NF κ B activity was assessed with the electrophoretic mobility shift assay. Media or lipopolysaccharide (LPS 1 μ g/mL) served as the negative and positive controls, respectively.

4. Discussion

We observed that a (1→3)- β -D linked glucan polymer composed of seven glucose subunits is the minimum binding ligand for glucan pattern recognition receptors on a human monocyte cell line. The data also indicate that the heptaose glucan interacts non-selectively with at least two binding sites. The heptaose glucan did not stimulate macrophage NF κ B nuclear binding activity or increase survival in the CLP model, despite the fact that the heptaose polymer is recognized by all macrophage glucan receptors. The oligosaccharides used in this study were derived from laminarin, a 7 700 g/mol glucan polymer [3]. We have previously demonstrated that laminarin is

recognized by one of the human monocyte glucan receptors using a cell-based binding/internalization assay [3]. The present data confirm and extend this observation. In both systems laminarin competed for ~60% of total binding with characteristics of a single binding site. However, the heptaose glucan polymer completely inhibited U937 membrane binding to a glucan-coated surface, indicating that the heptaose interacts with all available glucan-binding sites. This suggests that the basic (1→3)- β -D-glucan backbone structure, represented by the heptaose polymer, is specifically recognized by all available glucan receptors. However, more complex glucan polymers, such as laminarin, may be preferentially recognized by a subset of glucan receptors.

We [12] and others [11, 13, 35] have reported that various glucans will stimulate NF κ B, NF-IL6 activity, as well as pro-inflammatory and immunoregulatory cytokine production. This is consistent with the ability of these carbohydrate ligands to modulate innate immunity [10–13, 35, 36]. We examined the ability of the glucan oligosaccharides and laminarin to stimulate NF κ B activity in a cultured murine macrophage cell line. None of the oligosaccharides or laminarin increased macrophage NF κ B nuclear binding activity, when compared to lipopolysaccharide. The heptaose glucan did not alter survival in the murine model of polymicrobial sepsis. Thus, while the heptaose and laminarin are recognized by macrophage glucan receptors, they do not appear to be receptor agonists with regard to stimulating intracellular signaling pathways or induction of protective efficacy. It is not clear why larger more complex glucans will exert such activity [9], when the heptaose and laminarin will not. By way of example, glucans with molecular weights $\geq 14\ 100$ g/mol can effectively modulate innate immunity and protect against infectious challenge [34, 37]. There are reports that glucan polymers must cross-link membrane receptors in order to induce an immunomodulatory effect [38]. If this is true, then it may be that larger polymers are required in order to cross-link spatially separated receptors and alter immunocyte function. It is also possible that the lack of heptaose efficacy in the CLP model may be due to differences in pharmacokinetics, i.e. faster clearance, although even high-molecular-weight glucans, such as schizophyllan [39] and glucan phosphate (unpublished observation) are rapidly cleared from the vasculature. Furthermore, differences in pharmacokinetics would not play a role in tissue culture and could not explain the failure of the heptaose or laminarin to activate NF κ B in cultured monocytes. Consequently, our data tends to support the cross-linking model of activation.

The present data, when combined with previous reports [3, 18], indicate that polymer structure, molecular size and solution conformation have significant effects on the interaction of glucan ligands with their cognate receptors. The results of the present study suggest the intriguing possibility that the heptasaccharide glucan may have unique physicochemical characteristics when compared to the penta- and hexasaccharides. In an attempt to understand what differences there might be between the pentaose, hexaose and heptaose, we developed a simple molecular model of a single glucan polymer strand com-

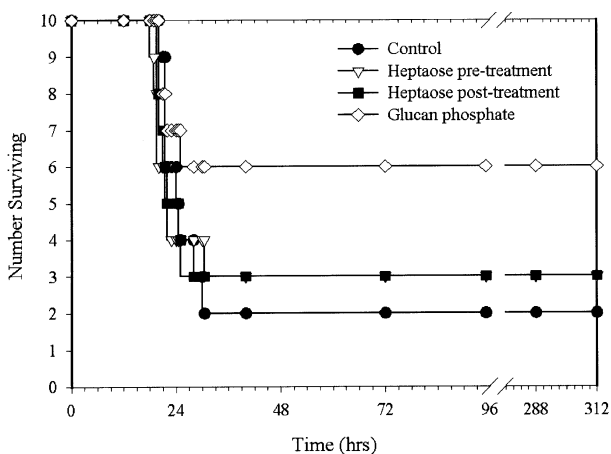


Figure 7. Pre- or post-treatment of mice with heptaose glucan (40 mg/kg) did not alter survival in ICR/HSD mice with CLP-induced polymicrobial sepsis. Control mice were treated with isovolumetric dextrose (5% w/v) and water. As a positive control, other groups of mice were treated i.p. (40 mg/kg) with glucan phosphate 1 h prior to induction of CLP.

posed of (1→3)-β-D-linked glucose subunits (not shown). The model was based on data derived from several sources. Kim et al. performed an in-depth structural characterization of the pentaose and heptaose [21]. They found the oligosaccharides to be linear, non-branched (1→3)-β-D-linked glucose polymers with Dp of 5 and 7, respectively [21]. We employed additional structural data derived from FT-NMR analysis of non-branched, single helical (1→3)-β-D-glucans [4, 21, 40], solution conformational data from linear scaling relationships [25] as well as small-angle X-ray and neutron scattering data for the same glucans [41]. Work on the model was also guided by the structural studies of Brant [42, 43], Marchessault [44, 45], Deslandes [46], Bluhm [47], Chanzy [48, 49], Sundarajan [50], McIntire [51] and colleagues. Because of the (1→3)-β intrachain linkage, glucan polymers assume a helical conformation [41–43]. We observed that six glucose subunits are required to complete one helical turn (data not shown). This indicates that the minimum binding subunit for the human monocyte glucan receptor requires one glucose subunit more than a full helical turn. Individual heptaose oligosaccharides are unlikely to form rigid helical structures in an aqueous environment at 37 °C. It is more likely that the polymers are semi-flexible or perturbed coils. Weis and Drickamer [52] have reviewed the structural basis for lectin-carbohydrate recognition. They suggest that selectivity and affinity of a binding site for complex polysaccharides may be due to the monosaccharide constituents interacting with multiple carbohydrate recognition domains (CRDs). In other words, a polymer of appropriate structure and size would have monosaccharides that are positioned to simultaneously interact with multiple CRDs, resulting in greater affinity or selectivity. We speculate that there are at least two planar CRDs in the human monocyte glucan receptors which interact with glucose monosaccharides at either end of the heptaose polymer, thus constituting the minimum binding site. However, the heptaose and laminarin produced no biological response, suggesting that interaction with additional CRDs is a requirement for induction of biological activity.

In conclusion, the present data indicate that all available monocyte glucan receptors will recognize the basic (1→3)-β-D-glucan structure with approximately the same affinity. However, as the glucan polymer becomes more complex it appears to be preferentially recognized by one glucan receptor versus another. The data also indicate that interaction of glucan receptors with heptasaccharide or laminarin polymers is not sufficient for induction of immunobiological activity. We speculate that this is due to the molecular size of the polymer. Taken together, our data suggest that the mechanisms by which the innate immune system recognizes and responds to fungal cell wall carbohydrates is a very complex and multi-factorial process.

Acknowledgments

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