

Mechanism of Action

Citation	Abstract
<p>Liz M. Lavigne, Jorge E. Albina and Jonathan S. Reichner</p> <p>β-Glucan is a Fungal Determinant for Adhesion-Dependent Human Neutrophil Functions.</p> <p>Journal of Immunology. Dec 2006; 177: 8667 – 8675</p>	<p><i>Candida albicans</i> is a common cause of nosocomial infections whose virulence depends on the reversible switch from blastoconidia to hyphal forms. Neutrophils (or polymorphonuclear leukocytes (PMNs)) readily clear blastoconidia by phagocytosis, but filaments are too long to be ingested. Mechanisms regulating immune recognition and response to filamentous fungal pathogens are not well understood, although known risk factors for developing life-threatening infections are neutropenia or defects in the NADPH oxidase system. We show human PMNs generate a respiratory burst response to unopsonized hyphae. Ab specific for β-glucan, a major component of yeast cell walls, blocks this response, establishing β-glucan as a key molecular pattern recognized by PMNs in response to <i>C. albicans</i>. This study also elucidates recognition and signaling mechanisms used by PMNs in response to β-glucan under conditions where phagocytosis cannot occur. Human PMNs adhered to immobilized β-glucan and released an efficient plasma membrane respiratory burst. Ab blockade of the integrin complement receptor 3 (CD11b/CD18) significantly inhibited both of these functions. Furthermore, we show a role for p38 MAPK and actin but not protein kinase C in generating the respiratory burst to β-glucan. Taken together, results show that β-glucan in <i>C. albicans</i> hyphae is accessible to PMNs and sufficient to support an innate immune response.</p>
<p>Brian W. LeBlanc, Jorge E. Albina and Jonathan S. Reichner</p> <p>The Effect of PGG-Beta-Glucan on Neutrophil Chemotaxis in Vivo</p> <p>J Leukoc Biol. 2006 Apr; 79 (4:667-75). Epub 2006 Jan 13.</p> <p>PMID: 16415173 [PubMed - indexed for MEDLINE]</p>	<p>The β-glucans are long-chain polymers of glucose in β-(1,3)(1,6) linkages, which comprise the fungal cell wall and stimulate cells of the innate immune system. Previous in vitro studies have shown the ability of β-glucan to increase the chemotactic capacity of human neutrophils. The current study examined an in vivo correlate of that observation by testing the hypothesis that systemic β-glucan treatment would result in enhanced migration of neutrophils into a site of inflammation and improve antimicrobial function. A model of acute inflammation was used in which polyvinyl alcohol sponges were implanted subcutaneously into the dorsum of rats. Animals treated with β-glucan showed a $66 \pm 6\%$ and $186 \pm 42\%$ increase in wound cell number recovered 6 and 18 h postwounding, respectively. Increased migration did not correlate with increased chemoattractant content of wound fluid, alterations in neutrophil-induced loss of endothelial barrier function, or changes in neutrophil adhesion to endothelial cells. Systemic administration of SB203580 abrogated the enhanced migration by β-glucan without altering normal cellular entry into the wound. Studies also showed a priming effect for chemotaxis and respiratory burst in circulating neutrophils isolated from β-glucan-treated animals. Heightened neutrophil function took place without cytokine elicitation. Furthermore, β-glucan treatment resulted in a $169 \pm 28\%$ increase in neutrophil number and a $60 \pm 9\%$ decrease in bacterial load in the bronchoalveolar lavage fluid of <i>Escherichia coli</i> pneumonic animals. Taken together, these findings demonstrate that β-glucan directly affects the chemotactic capacity of circulating neutrophils through a p38 mitogen-activated protein kinase-dependent mechanism and potentiates antimicrobial host defense.</p>

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<p>Tsikitis V, Albina J, Reichner J</p> <p>β-Glucan Affects Leukocyte Navigation in a Complex Chemotactic Gradient</p> <p><i>Surgery.</i> 2004 Aug;136(2):384-9.</p>	<p>Background Polymorphonuclear leukocytes (PMNs) must traverse endogenous chemotactic gradients (interleukin 8 [IL-8]) before reaching target chemoattractants (fMLP [N- formylmethionine-leucine-phenylalanine], C5a) produced at a site of bacterial infection. Complement receptor 3 (CR3; CD11b/CD18) contains 2 distinct binding sites, one that mediates adhesion and a lectin-like domain (LLD) that binds polysaccharides of microbial origin. This laboratory previously reported an increase in the chemotactic capacity of PMNs toward fMLP upon ligation of the CR3 LLD with beta-glucan, a CR3 agonist. Current studies sought to determine the effect of β-glucan on PMN navigation toward other chemoattractants alone and in a competing chemotactic environment.</p> <p>Methods Migration was assessed by serum agarose overlay with the use of chambered slides containing or not, β-glucan. Migration of human PMNs at 37 degrees C for 2 hours was evaluated toward C5a or IL-8 alone and in competing gradients. Selected groups were treated with anti-CR3-blocking antibodies. The number of chemotactic cells was quantified by microscopy.</p> <p>Results β-glucan significantly enhanced chemotaxis toward C5a and suppressed that toward IL-8 in a CR3-dependent fashion. In the competing chemotactic gradient assays (C5a vs IL-8), β-glucan further enhanced migration toward C5a while not affecting that toward IL-8.</p> <p>Conclusions β-glucan selectively upregulates PMN chemotaxis toward C5a while suppressing chemotaxis toward IL-8. (<i>Surgery</i> 2004;136:384-9.)</p> <p>From the Department of Surgery, Rhode Island Hospital and Brown Medical School, Providence, RI</p>
<p>Xia, Y., Borland, G., Huang, J., Mizukami, I., Petty, H. R., Todd, R. F., III, and Ross, G. D.</p> <p>Function of the lectin domain of Mac-1/complement receptor type 3 (CD11b/CD18) in regulating neutrophil adhesion.</p> <p><i>J. Immunol.</i>, 169:6417-6426, 2002.</p> <p>PMID: 12444150 [PubMed - indexed for MEDLINE]</p>	<p>A lectin function within CD11b mediates both cytotoxic priming of Mac-1/complement receptor type 3 (CR3) by beta-glucan and the formation of transmembrane signaling complexes with GPI-anchored glycoproteins such as CD16b (FcgammaRIIIb). A requirement for GPI-anchored urokinase plasminogen activator receptor (uPAR; CD87) in neutrophil adhesion and diapedesis has been demonstrated with uPAR-knockout mice. In this study, neutrophil activation conditions generating high-affinity (H-AFN) or low-affinity (L-AFN) beta(2) integrin adhesion were explored. A role for the Mac-1/CR3 lectin domain and uPAR in mediating H-AFN or L-AFN adhesion was suggested by the inhibition of Mac-1/CR3-dependent adhesion to ICAM-1 or fibrinogen by beta-glucan or anti-uPAR. The formation of uPAR complexes with Mac-1/CR3 activated for L-AFN adhesion was demonstrated by fluorescence resonance energy transfer. Conversely, Jurkat cell LFA-1 H-AFN-adhesion to ICAM-1 was not associated with uPAR/LFA-1 complexes, any requirement for GPI-anchored glycoproteins, or inhibition by beta-glucan. A single CD11b lectin site for beta-glucan and uPAR was suggested because the binding of either beta-glucan or uPAR to Mac-1/CR3 selectively masked two CD11b epitopes adjacent to the transmembrane domain. Moreover, treatment with phosphatidylinositol-specific phospholipase C that removed GPI-anchored proteins increased CD11b-specific binding of (125)I-labeled beta-glucan by 3-fold and this was reversed with soluble recombinant uPAR. Conversely, neutrophil activation for generation of Mac-1/CR3/uPAR complexes inhibited CD11b-dependent binding of (125)I-labeled beta-glucan by 75%. These data indicate that the same lectin domain within CD11b regulates both the cytotoxic and adhesion functions of Mac-1/CR3.</p>

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<p>Xia, Y. and Ross, G. D.</p> <p>Generation of recombinant fragments of CD11b expressing the functional β-glucan-binding lectin site of CR3 (CD11b/CD18).</p> <p><i>J. Immunol.</i>, 162:7285-7293, 1999.</p> <p>PMID: 10358177 [PubMed - indexed for MEDLINE]</p>	<p>CR3 (Mac-1; αMβ2 integrin) functions as both a receptor for the opsonic iC3b fragment of C3 triggering phagocytosis or cytotoxicity and an adhesion molecule mediating leukocyte diapedesis. Recent reports have suggested that a CR3 lectin site may be required for both cytotoxic responses and adhesion. Cytotoxic responses require dual recognition of iC3b via the I domain of CD11b and specific microbial surface polysaccharides (e.g., beta-glucan) via a separate lectin site. Likewise, adhesion requires a lectin-dependent membrane complex between CR3 and CD87. To characterize the lectin site further, a recombinant baculovirus (rBv) system was developed that allowed high level expression of rCD11b on membranes and in the cytoplasm of Sf21 insect cells. Six rBv were generated that contained truncated cDNA encoding various CD11b domains. Immunoblotting of rBv-infected Sf21 cells showed that some native epitopes were expressed by five of six rCD11b fragments. Lectin activity of rCD11b proteins was evaluated by both flow cytometry with beta-glucan-FITC and radioactive binding assays with [¹²⁵I]beta-glucan. Sf21 cells expressing rCD11b that included the C-terminal region, with or without the I-domain, exhibited lectin activity that was inhibited by unlabeled beta-glucan or anti-CR3 mAbs. The smallest rCD11b fragment exhibiting lectin activity included the C-terminus and part of the divalent cation binding region. The beta-glucan binding affinities of the three C-terminal region-containing rCD11bs expressed on Sf21 cell membranes were not significantly different from each other and were similar to that of neutrophil CR3. These data suggest that the lectin site may be located entirely within CD11b, although lectin site-dependent signaling through CD18 probably occurs with the heterodimer.</p>
<p>Patchen ML, Liang J, Vaudrain T, Martin T, Melican D, Zhong S, Stewart M, Quesenberry PJ.</p> <p>Mobilization of peripheral blood progenitor cells by Betafectin PGG-Glucan alone and in combination with granulocyte colony-stimulating factor.</p> <p><i>Stem Cells.</i> 1998;16(3):208-17.</p> <p>PMID: 9617896 [PubMed - indexed for MEDLINE]</p>	<p>Betafectin PGG-Glucan, a novel beta-(1,6) branched beta-(1,3) glucan purified from the cell walls of <i>Saccharomyces cerevisiae</i>, has been shown to synergize with myeloid growth factors in vitro and to enhance hematopoietic recovery in myelosuppressed mice and primates. Here we report that PGG-Glucan is also capable of mobilizing peripheral blood progenitor cells (PBPC). PGG-Glucan (0.5 mg/kg to 16 mg/kg) was administered intravenously to C3H/HeN male mice and blood collected at times ranging from 30 min to seven days after injection. Based on granulocyte-macrophage colony-forming cell (GM-CFC) levels, peak mobilization occurred 30 min after a 2 mg/kg PGG-Glucan dose. At this time GM-CFC numbers in PGG-Glucan-treated mice were approximately fourfold greater than in saline-treated control mice. A second, smaller wave of GM-CFC mobilization (approximately twofold increase) also occurred on days 4 and 5 after PGG-Glucan treatment. Mobilization was not associated with the induction of alpha-chemokines, which have recently been reported to induce rapid progenitor cell mobilization. Competitive repopulation experiments performed in irradiated female C3H/HeN mice revealed that, at three months after transplantation, more male DNA was present in bone marrow, splenic, and thymic tissues from animals transplanted with cells obtained from mice 30 min after a 2 mg/kg PGG-Glucan dose than in tissues from animals transplanted with cells obtained from saline-treated mice. Additional experiments evaluated the mobilization effects of PGG-Glucan (2 mg/kg) administered to mice which had been pretreated for three consecutive days with G-CSF (125 microg/kg/day). When blood was collected 30 min after PGG-Glucan treatment, the number of GM-CFC mobilized in combination-treated mice was additive between the number mobilized in mice treated with G-CSF alone and the number mobilized in mice treated with PGG-Glucan alone. These studies demonstrate that: A) PGG-Glucan can rapidly mobilize PBPC; B) the kinetic pattern of PGG-Glucan-induced mobilization is different from that of the CSFs; C) the reconstitutive potential of PGG-Glucan mobilized cells is greater than that of steady-state PBPC, and D) PGG-Glucan can enhance G-CSF-mediated PBPC mobilization.</p>

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<p>Thornton, B. P., Větvíčka, V., Pitman, M., Goldman, R. C., and Ross, G. D.</p> <p>Analysis of the sugar specificity and molecular location of the β-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18).</p> <p><i>J. Immunol.</i>, 156:1235-1246, 1996.</p> <p>PMID: 8558003 [PubMed - indexed for MEDLINE]</p>	<p>Zymosan, the cell wall from <i>Saccharomyces cerevisiae</i>, was reported to be a macrophage activator through its beta-glucan over 30 yr ago. Nevertheless, the identity of the beta-glucan receptor has been controversial. This study showed that the alpha M beta 2-integrin, CR3 (Mac-1, CD11b/CD18) served as the beta-glucan receptor through one or more lectin sites located outside of the CD11b I-domain that contains the binding sites for iC3b, ICAM-1, and fibrinogen. Sugar specificity, analyzed with FITC-labeled soluble polysaccharides and flow cytometry, showed CR3-specific staining with several pure beta-glucans but not with alpha-mannan. However, a 10-kDa soluble zymosan polysaccharide (SZP) with high affinity (6.7×10^{-8} M) for CR3 consisted largely of mannose and approximately 5% glucose. Binding of either SZP-FITC or beta-glucan-FITC to CR3 was blocked not only by pure beta-glucans from yeast, mushroom, seaweed, or barley, but also by N-acetyl-D-glucosamine (NADG), alpha- or beta-methylmannoside, and alpha- or beta-methyl-glucoside. SZP-FITC and beta-glucan-FITC stained all leukocyte types similarly to anti-CR3-FITC, and polysaccharide-FITC staining was inhibited $> \text{ or } = 95\%$ by unlabeled anti-CR3. SZP-FITC staining of cells expressing recombinant chimeras between CR3 and CR4 (p150,95, CD11c/CD18) suggested that both the divalent cation-binding region of CD11b and the region C-terminal to it may regulate binding of polysaccharides to CR3. Unlabeled SZP or beta-glucan also blocked CR3 staining by 11 mAb to C-terminal domain epitopes of CD11b but had no effect on staining by mAb directed to the I-domain. In conclusion, CR3 serves as the leukocyte beta-glucan receptor through a cation-independent lectin site located C-terminal to the I-domain of CD11b. Its sugar specificity is broader than originally appreciated, allowing it to react with certain polysaccharides containing mannose or NADG, as well as glucose.</p>
<p>Konopski Z, Rasmussen LT, Seljelid R, Eskeland T.</p> <p>Phagocytosis of beta-1,3-D-glucan-derivatized microbeads by mouse peritoneal macrophages involves three different receptors.</p> <p><i>Scand J Immunol.</i> 1991 Mar;33(3): 297-306.</p> <p>PMID: 1849314 [PubMed - indexed for MEDLINE]</p>	<p>Intraperitoneal injection of beta-1,3-D-glucan coupled to the surface of monodisperse methacrylate microbeads improves the resistance against bacterial infections in mice, while methacrylate microbeads alone do not. The effect of the glucan-derivatized microbeads (GDM) is considered to be mediated through peritoneal macrophages. We show that both GDM and the underivatized methacrylate microbeads (UDM) treated with normal serum were rapidly bound and phagocytized by mouse peritoneal macrophages in vitro. We found that both complement and fibronectin opsonized the beads and were responsible for the uptake. Treatment of microbeads with serum lacking fibronectin and complement activity still gave some uptake of GDM, but not uptake of UDM. The uptake of GDM was similar to the uptake of untreated GDM and was inhibited by pretreatment of macrophages with soluble beta-1,3-D-glucan. Our conclusion is that GDM and UDM intraperitoneally bind fibronectin and C3 through activation of the alternative pathway of complement. This leads to their phagocytosis by macrophages through fibronectin and complement receptors. GDM are also internalized via beta-glucan receptors. We present the hypothesis that the beta-glucan receptors on peritoneal macrophages account for the protective effect of GDM in intraperitoneal bacterial infections.</p>