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Yeast Beta 1,3/1,6 Glucan	
Citation	**Abstract**
Daniel J. Allendorf, Jun Yan, Gordon D. Ross, Richard D. Hansen, Jarek T. Baran, Krishnaprasad Subbarao, Li Wang, and Bodduluri Haribabu **C5a-Mediated Leukotriene B4-Amplified Neutrophil Chemotaxis Is Essential in Tumor Immunotherapy Facilitated by Anti-Tumor Monoclonal Antibody and β-Glucan** The Journal of Immunology, 2005, 174: 7050–7056. PMID: 15905548 [PubMed - in process]	Intravenous and orally administered β-glucans promote tumor regression and survival by priming granulocyte and macrophage C receptor 3 (CR3, iC3bR and CD11b/CD18) to trigger the cytotoxicity of tumor cells opsonized with iC3b via anti-tumor Abs. Despite evidence for priming of macrophage CR3 by oral β-glucan in vivo, the current study in C57BL/6 and BALB/c mice showed that granulocytes were the essential killer cells in mAb- and oral β-glucan-mediated tumor regression, because responses were absent in granulocyte-depleted mice. Among granulocytes, neutrophils were the major effector cells, because tumor regression did not occur when C5a-dependent chemotaxis was blocked with a C5aR antagonist, whereas tumor regression was normal in $C3aR^{-}/^{-}$ mice. Neutrophil recruitment by C5a in vivo required amplification via leukotriene B4, because both C5a-mediated leukocyte recruitment into the peritoneal cavity and tumor regression were suppressed in leukotriene B4R-deficient ($BLT\text{-}1^{-}/^{-}$) mice.
Jun Yan, Daniel J Allendorf & Brian Brandley **Yeast Whole Glucan Particle ß-Glucan in Conjunction with Anti-tumour Monoclonal Antibodies to Treat Cancer** Expert Opinion on Biological Therapy May 2005, Vol. 5, No. 5, Pages 691-702 PMID: 15934844 [PubMed - in process]	Beta-glucans, biological response modifiers (BRMs) derived from the cell walls of yeast and other sources, have been demonstrated to prime leukocyte complement receptor 3 (CR3), thus enabling these cells to kill tumours opsonised with complement fragment iC3b. Many tumours activate complement via the classical pathway mediated by antitumour monoclonal antibodies (mAbs) or natural antibodies. Studies into the cellular and molecular mechanisms of action have demonstrated that orally administrated yeast β-glucans are ingested and processed by macrophages. These macrophages secrete the active moiety that primes neutrophil CR3 to kill iC3b-opsonised tumour cells. Extensive studies in preclinical animal tumour models have demonstrated the efficacy of combined oral particulate yeast β-glucan with antitumour mAb therapy in terms of tumour regression and long-term survival. It is proposed that the addition of β-glucan will further improve the clinical therapeutic efficacy of antitumour mAbs in cancer patients.
Feng Hong, Jun Yan, Jarek T. Baran, Daniel J. Allendorf, Richard D. Hansen, Gary R. Ostroff, Pei Xiang Xing, Nai-Kong V. Cheung, and Gordon D. Ross **Mechanism by Which Orally Administered β-1,3-Glucans Enhance the Tumoricidal Activity of Antitumor Monoclonal Antibodies in Murine Tumor Models** The Journal of Immunology, 2004, 173: 797–806. PMID: 15240666 [PubMed - indexed for MEDLINE]	Antitumor mAb bind to tumors and activate complement, coating tumors with iC3b. Intravenously administered yeast β-1,3; 1,6-glucan functions as an adjuvant for antitumor mAb by priming the inactivated C3b (iC3b) receptors (CR3; CD11b/CD18) of circulating granulocytes, enabling CR3 to trigger cytotoxicity of iC3b-coated tumors. Recent data indicated that barley β-1,3; 1,4-glucan given orally similarly potentiated the activity of antitumor mAb, leading to enhanced tumor regression and survival. This investigation showed that orally administered yeast β-1,3;1,6-glucan functioned similarly to barley β-1,3;1,4-glucan with antitumor mAb. With both oral β-1,3-glucans, a requirement for iC3b on tumors and CR3 on granulocytes was confirmed by demonstrating therapeutic failures in mice deficient in C3 or CR3. Barley and yeast β-1,3-glucan were labeled with fluorescein to track their oral uptake and processing in vivo. Orally administered β-1,3-glucans were taken up by macrophages that transported them to spleen, lymph nodes, and bone marrow. Within the bone marrow, the macrophages degraded the large β-1,3-glucans into smaller soluble β-1,3-glucan fragments that were taken up by the CR3 of marginated granulocytes. These granulocytes with CR3-bound β-1,3-glucan-fluorescein were shown to kill iC3b-opsonized tumor cells following their recruitment to a site of complement activation resembling a tumor coated with mAb.

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Hong, F., Hansen, R. D., Yan, J., Allendorf, D. J., Baran, J. T., Ostroff, G. R., and Ross, G. D. **β-Glucan Functions as an Adjuvant for Monoclonal Antibody Immunotherapy by Recruiting Tumoricidal Granulocytes as Killer Cells** Cancer Research, 63(24):9023-31, Dec. 15, 2002. PMID: 14695221 [PubMed – indexed for Medline]	The tumor-killing mechanisms available to monoclonal antibodies (mAbs; *e.g*. antagonism of growth factor receptors, antibody-dependent cell-mediated cytotoxicity) limit efficacy. Previous studies suggested that i.v. β-glucan might function as an adjuvant for antitumor mAbs. β-glucan had been shown to function via the iC3b-receptor complement receptor 3 (CR3; CD11b/CD18) thereby enhancing leukocyte killing of tumor cells coated with iC3b via naturally occurring antitumor antibodies. Therapy with β-Glucans was limited by levels of natural antibodies and by tumor escape through elimination of antigen-positive cells. Accordingly, it was hypothesized that β-glucan responses could be improved by combined administration with antitumor mAbs. Five tumor models were explored in BALB/c or C57B1/6 mice using tumors that expressed either high levels of naturally occurring antigens (*e.g*. G_{D2} ganglioside) or recombinant human MUC1. In comparison with antitumor mAb or β-glucan alone, combined treatment with mAb plus β-Glucan produced significantly greater tumor regression in all models that included mammary, s.c., and hepatic tumors. Tumor-free survival only occurred in models that incorporated stable expression of the target antigen. β-Glucan enhancement of the mAb tumoricidal response did not occur in mice deficient in either leukocyte CR3 ($CD11b^{-/-}$) or serum C3, confirming the requirement for CR3 on leukocytes and iC3b on tumors. Granulocytes appeared to be primarily responsible for tumoricidal activity, because β-Glucan therapeutic responses did not occur in granulocyte-depleted mice. These data suggest that they therapeutic efficacy of mAbs known to activate complement (e.g. Herceptin, Rituxan and Erbitux) could be significantly enhanced if they were combined with β-Glucan.
Xia, Y., Borland, G., Huang, J., Mizukami, I., Petty, H. R., Todd, R. F., III, and Ross, G. D. **Function of the lectin domain of Mac-1/complement receptor type 3 (CD11b/CD18) in regulating neutrophil adhesion.** *J. Immunol.,* 169:6417-6426, 2002. PMID: 12444150 [PubMed - indexed for MEDLINE]	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.

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Vetvicka, V, Terayama K, Mandeville R, Brousseau P, Kournikakis B, Ostroff G **Orally-administered Yeast β1,3-glucan prophylactically protects against anthrax infection and cancer in mice** *Journal of the American Nutraceutical Association.* Vol. 5, No. 2, Spring 2002.	β1,3-glucans from various bacterial, mushroom, yeast, and cereal sources have been established as immunomodulators. In the present paper we demonstrate that orally-administered yeast β1,3-glucan had significant effects as a prophylactic treatment to reduce the mortality of anthrax infection in mice. In addition, the same type of treatment also inhibited the growth of metastatic cancer cells *in vivo.* The mechanism of action involves the stimulation of three important cytokines: IL-2, IFN-γ, and TNF-α. These results provide preclinical evidence for the beneficial effects of orally-administered yeast β1,3-glucan.
Yoshino, S., T. Tabata, S. Hazama, N. Iizuka, K. Yamamoto, M. Hirayama, A. Tangoku, and M. Oka. **Immunoregulatory effects of the antitumor polysaccharide lentinan on Th1/Th2 balance in patients with digestive cancers.** *Anticancer Res*. 20:4707-4711. 2000 PMID: 11205205 [PubMed - indexed for MEDLINE]	**Background**: Recent studies demonstrated that patients with advanced cancer may have impaired cell-mediated immunity caused by an imbalance between Th1 and Th2 responses. We evaluated the ability of lentinan (LNT) to modulate Th1 and Th2 responses in patients with digestive cancers. **Methods**: Peripheral blood samples were collected preoperatively from 28 patients with digestive cancers before and after intravenous administration of LNT (2 mg x 3 times/week). The proportions of CD4+ T-cells producing intracellular cytokines were determined with flow cytometry. **Results**: After LNT treatment, CD4+ IFN-gamma+ T-cell percentages increased significantly ($p < 0.05$), whereas CD4+ IL-4+ T-cell and CD4+ IL-6+ T-cell percentages decreased significantly ($p < 0.02$). No significant change occurred in proportions of CD4+ IL-10+ T-cells. The after/before LNT treatment percentages ratio of CD4+ IFN-gamma+ T-cells correlated negatively with that of CD4+ IL-4+ T-cells ($p < 0.01$). The after/before treatment percentage ratio of CD4+ IL-4+ T-cells correlated positively with that of CD4+ IL-6+ T-cells ($p < 0.05$). CONCLUSION: LNT apparently can cancel Th2-dominant condition in patients with digestive cancers and may improve the balance between Th1 and Th2.
Tokunaka K, Ohno N, Adachi Y, Tanaka S, Tamura H, Yadomae T. **Immunopharmacological and immunotoxicological activities of a water-soluble (1-->3)-beta-D-glucan, CSBG from Candida spp.** *Int J Immunopharmacol.* 2000 May;22(5):383-94. PMID: 10708886 [PubMed - indexed for MEDLINE]	We have established a convenient, two-step procedure to solubilize the yeast cell wall (1-->3)-beta-D-glucan using the combination of NaClO oxidation and DMSO extraction. Candida soluble beta-D-glucan (CSBG) was mainly composed of a linear beta-1,3 glucan with a linear beta-1,6-glucan moiety. In this study, we screened for several immunopharmacological activities of CSBG and found the following activities: (1) interleukin-6 synthesis of macrophages in vitro; (2) antagonistic effect for zymosan mediated-tumor necrosis factor synthesis of macrophages; (3) augmentation for lipopolysaccharide mediated tumor necrosis factor and nitrogen oxide syntheses of macrophages; (4) activation of alternative pathway of complement; (5) hematopoietic response on cyclophosphamide induced leukopenia; (6) the antitumor effect on ascites form tumor; (7) Enhanced vascular permeability; (8) priming effect on lipopolysaccharide triggered TNF-alpha synthesis; and (9) adjuvant effect on antibody production. These results strongly suggested that CSBG possessed various immunopharmacological activity.

Ross GD, Vetvicka V, Yan J, Xia Y, Vetvickova J. **Therapeutic intervention with complement and beta-glucan in cancer.** *Immunopharmacology*. 1999 May; 42(1-3):61-74. Review. PMID: 10408367 [PubMed - indexed for MEDLINE]	Complement (C) has two major effector systems available for host defense. The membrane attack complex (MAC) generated from components C5-C9 can form membrane-penetrating lesions that lead to cell death by causing a rapid loss of cytoplasmic components. The MAC is only effective against pathogens with outer phospholipid membranes, and cannot kill gram-positive bacteria or yeast whose membranes are protected by cell walls. The most important effector mechanism of C is the opsonization of microbial pathogens with the serum protein C3 that leads to their high avidity attachment to the C3-receptors of phagocytic cells. Pathogens that activate complement are first coated with the C3b fragment of C3, which is rapidly proteolyzed into the iC3b fragment by serum factor I. These iC3b fragments serve to promote the high avidity attachment of the 'iC3b-opsonized' pathogens to the iC3b-receptors (CR3, CD11b/CD18) of phagocytic cells and natural killer (NK) cells, stimulating phagocytosis and/or cytotoxic degranulation. Host cells, including neoplastic tumor cells, have been endowed with natural mechanisms for self-protection against both the MAC and the cytotoxic activation of CR3. This review discusses a novel type of immunotherapy for cancer that uses soluble yeast beta-glucan to override the normal resistance of iC3b-opsonized tumor cells to the cytotoxic activation of phagocyte and NK cell CR3, allowing this important effector mechanism of the C system to function against tumor cells in the same way that it normally functions against bacteria and yeast. Moreover, the cytotoxic activation of beta-glucan-primed NK cell CR3 by iC3b-opsonized tumors is shown to be accompanied by a tumor-localized secretion of the cytokines TNFalpha, IFNalpha, IFNgamma, and IL-6.
Yan, J., Větvička, V., Xia, Y., Coxon, A., Carroll, M.C., Mayadas, T.N., and Ross, G.D. **β-Glucan, a "specific" biologic response modifier that uses antibodies to target tumors for recognition by complement receptor 3 (CD11b/CD18).** *J. Immunol.,* 163:3045-3052, 1999. PMID: 10477568 [PubMed - indexed for MEDLINE]	Beta-Glucans were identified 36 years ago as a biologic response modifier that stimulated tumor rejection. In vitro studies have shown that beta-glucans bind to a lectin domain within complement receptor type 3 (CR3; known also as Mac-1, CD11b/CD18, or alphaMbeta2-integrin, that functions as an adhesion molecule and a receptor for factor I-cleaved C3b, i.e., iC3b) resulting in the priming of this iC3b receptor for cytotoxicity of iC3b-opsonized target cells. This investigation explored mechanisms of tumor therapy with soluble beta-glucan in mice. Normal mouse sera were shown to contain low levels of Abs reactive with syngeneic or allogeneic tumor lines that activated complement, depositing C3 onto tumors. Implanted tumors became coated with IgM, IgG, and C3, and the absent C3 deposition on tumors in SCID mice was reconstituted with IgM or IgG isolated from normal sera. Therapy of mice with glucan- or mannan-rich soluble polysaccharides exhibiting high affinity for CR3 caused a 57-90% reduction in tumor weight. In young mice with lower levels of tumor-reactive Abs, the effectiveness of beta-glucan was enhanced by administration of a tumor-specific mAb, and in SCID mice, an absent response to beta-glucan was reconstituted with normal IgM or IgG. The requirement for C3 on tumors and CR3 on leukocytes was highlighted by therapy failures in C3- or CR3-deficient mice. Thus, the tumoricidal function of CR3-binding polysaccharides such as beta-glucan in vivo is defined by natural and elicited Abs that direct iC3b deposition onto neoplastic cells, making them targets for circulating leukocytes bearing polysaccharide-primed CR3. Therapy fails when tumors lack iC3b, but can be restored by tumor-specific Abs that deposit iC3b onto the tumors.

Xia, Y. and Ross, G. D. **Generation of recombinant fragments of CD11b expressing the functional β-glucan-binding lectin site of CR3 (CD11b/CD18).** *J. Immunol*., 162:7285-7293, 1999. PMID: 10358177 [PubMed - indexed for MEDLINE]	CR3 (Mac-1; alphaMbeta2 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.
Xia, Y., Větvička, V., Yan, J., Hanikýřová, M., Mayadas, T. N., and Ross, G. D. **The β-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells.** *J. Immunol.,* 162:2281-2290, 1999 PMID: 9973505 [PubMed - indexed for MEDLINE]	Mouse leukocyte CR3 (Mac-1, alphaMbeta2 integrin) was shown to function as a receptor for beta-glucans in the same way as human CR3. Soluble zymosan polysaccharide (SZP) or pure beta-glucans labeled with FITC or 125I bound in a saturable and reversible manner to neutrophils, macrophages, and NK cells. This lectin activity was blocked by anti-CD11b mAb M1/70 or 5C6 and did not occur with leukocytes from CR3-/- (CD11b-deficient) mice. SZP preparations containing primarily mannose or glucose bound to CR3, and the binding of 125I-labeled beta-glucan to CR3 was competitively inhibited by beta-glucans from barley or seaweed, but not by yeast alpha-mannan. Also, as with human CR3, the lectin site of mouse CR3 was inhibited by alpha- or beta-methylglucoside (but not D-glucose), alpha- or beta-methylmannoside, and N-acetyl-D-glucosamine. Phagocytosis of zymosan and serum-opsonized zymosan was partially inhibited by anti-CR3 and was reduced to <40% of normal with leukocytes from CR3-/- mice. As with neutrophils from patients with CD18 deficiency, neutrophils from CR3-/- mice exhibited no phagocytosis of particulate beta-glucan. SZP or beta-glucans primed CR3 of neutrophils, macrophages, and NK cells for cytotoxicity of iC3b-opsonized tumor cells that otherwise did not trigger killing. beta-Glucan priming for cytotoxicity was inhibited by anti-CR3 and did not occur with leukocytes from CR3-/- mice. The primed state of macrophage and NK cell CR3 remained detectable for 18 to 24 h after pulsing with beta-glucans. The similarity of mouse and human CR3 in response to beta-glucans highlights the utility of mouse tumor models for development of therapeutic beta-glucans.

Větvička, V., Thornton, B. P., Wieman, T. J., and Ross, G. D. **Targeting of NK cells to mammary carcinoma via naturally occurring tumor cell-bound iC3b and β-glucan-primed CR3 (CD11b/CD18).** *J. Immunol.,* 159:599-605, 1997. PMID: 9218574 [PubMed - indexed for MEDLINE]	Previous reports have suggested that malignant cells frequently generate a humoral immune response that is ineffective in tumor destruction. Despite coating tumors with IgM and IgG that activate the C system via the classical pathway, normal membrane regulators of C (e.g., membrane cofactor protein and CD59) prevent cytotoxicity. Moreover, C3 deposition on tumors does not result in cytotoxic recognition by phagocytes or NK cells bearing C3 receptors capable of mediating destruction of C3-opsonized bacteria or yeast. The current investigation showed that freshly excised mammary tumors bore IgM, IgG, and C3 detectable by flow cytometry. Normal sera contained natural IgM and IgG Abs reactive with breast tumor cell lines, and IgG Ab titers were increased in patients with breast cancer. Breast tumor cell lines incubated in normal serum from AB+ individuals activated the classical, but not the alternative, pathway of C and became coated with C3. Despite exhibiting membrane-bound C3, serum-opsonized breast tumor cell lines were not killed by CR3 (CD11b/CD18)-bearing NK cells. Priming of NK cell CR3 with small soluble yeast beta-glucan polysaccharides enabled CR3-dependent killing of these same C3-bearing tumor cell lines. Tests of mammary carcinoma cells from freshly excised tumors demonstrated that they also bore sufficient amounts of opsonic C3 for cytotoxic recognition by NK cells bearing polysaccharide-primed CR3, whereas they were largely resistant to NK cells bearing unprimed CR3. This study demonstrates the potential utility of using naturally occurring opsonic C3 on tumor cells for specific immunotherapeutic targeting by NK cells and phagocytes bearing polysaccharide-primed CR3.
Větvička, V., Thornton, B. P., and Ross, G. D. **Soluble β-glucan polysaccharide binding to the lectin site of neutrophil or NK cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells.** *J. Clin. Invest.,* 98:50-61, 1996. PMID: 8690804 [PubMed - indexed for MEDLINE]	When phagocyte CR3 binds to iC3b on bacteria or yeast, phagocytosis and degranulation are triggered because of simultaneous recognition of iC3b via a CD11b I-domain binding site and specific microbial polysaccharides via a lectin site located COOH-terminal to the I-domain. By contrast, when phagocyte or natural killer (NK) cell CR3 adheres to iC3b on erythrocytes or tumor cells that lack CR3-binding membrane polysaccharides, neither lysis nor cytotoxicity are stimulated. This investigation showed that soluble CR3-specific polysaccharides such as beta-glucan induced a primed state of CR3 that could trigger killing of iC3b-target cells that were otherwise resistant to cytotoxicity. Anti-CR3 added before sugars prevented priming, whereas anti-CR3 added after sugars blocked primed CR3 attachment to iC3b-targets. Polysaccharide priming required tyrosine kinase(s) and a magnesium-dependent conformational change of the I-domain that exposed the CBRM1/5 activation epitope. Unlike LPS or cytokines, polysaccharides did not up-regulate neutrophil CR3 expression nor expose the mAb 24 reporter epitope representing the high affinity ICAM-1-binding state. The current data apparently explain the mechanism of tumoricidal beta-glucans used for immunotherapy. These polysaccharides function through binding to phagocyte or NK cell CR3, priming the receptor for cytotoxicity of neoplastic tissues that are frequently targeted with iC3b and sparing normal tissues that lack iC3b.

Thornton, B. P., Větvička, V., Pitman, M., Goldman, R. C., and Ross, G. D. **Analysis of the sugar specificity and molecular location of the β-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18).** *J. Immunol.,* 156:1235-1246, 1996. PMID: 8558003 [PubMed - indexed for MEDLINE]	Zymosan, the cell wall from Saccharomyces cerevisiae, 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 x 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 > 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.
Sveinbjornsson B, Olsen R, Seternes OM, Seljelid R. **Macrophage cytotoxicity against murine meth A sarcoma involves nitric oxide-mediated apoptosis.** *Biochem Biophys Res Commun.* 1996 Jun 25;223(3):643-9. PMID: 8687449 [PubMed - indexed for MEDLINE]	We have studied the cytotoxic effect of stimulated macrophages on Meth A tumor cells in vitro. When stimulated with interferon-gamma and soluble beta-1,3-D-glucan, macrophages exerted cytotoxicity towards syngeneic Meth A tumor cells. This cytotoxicity was associated with a high level of nitric oxide production. Both cell death and nitric oxide production were significantly inhibited by the addition of aminoguanidine, a specific inhibitor of inducible nitric oxide synthase (iNOS), to the culture medium. The cytotoxic effect was accompanied by internucleosomal cleavage of DNA as shown by electrophoresis and DNA fragmentation assay.