

Citation	Abstract
<p>Carolina Salvador, Bing Li, Richard Hansen, Daniel E. Cramer, Maiying Kong and Jun Yan</p> <p><b>Yeast-Derived <math>\beta</math>-Glucan Augments the Therapeutic Efficacy Mediated by Anti-Vascular Endothelial Growth Factor Monoclonal Antibody in Human Carcinoma Xenograft Models</b></p> <p>Clinical Cancer Research 2008;1239 14(4) February 15, 2008</p>	<p>Bevacizumab is a recombinant IgG1 humanized monoclonal antibody against vascular endothelial growth factor (VEGF). Its proposed mechanism of action is independent of immune effector functions. Many human carcinomas not only secrete VEGF but also express membrane-bound VEGF. In addition, VEGF receptors are expressed on tumor cells. It is hypothesized that bevacizumab could bind membrane-bound VEGF or VEGF-VEGF receptor complexes on tumors, thereby initiating potential immunologic consequences. We previously showed that yeast-derived <math>\beta</math>-glucan functions with antitumor antibodies that activate complement to recruit complement receptor 3-expressing leukocytes capable of mediating complement receptor 3-dependent cellular cytotoxicity of tumors opsonized with iC3b. In the current study, the therapeutic efficacy mediated by combining bevacizumab with yeast-derived <math>\beta</math>-glucan was studied in human carcinoma xenograft models. Results: Human ovarian carcinoma SKOV-3 cells expressed membrane-bound VEGF both in vitro and in vivo. Bevacizumab was bound to membrane-bound VEGF, activated complement, and synergized with <math>\beta</math>-glucan to elicit cellular cytotoxicity in vitro. In vivo study showed that <math>\beta</math>-glucan could significantly augment the therapeutic efficacy mediated by bevacizumab. Conclusions: Yeast-derived <math>\beta</math>-glucan can synergize with anti-VEGF monoclonal antibody bevacizumab for the treatment of cancer with membrane-bound VEGF expression.</p>
<p>Bing Li, Daniel J. Allendorf, Richard Hansen, Jose Marroquin, Chuanlin Ding, Daniel E. Cramer and Jun Yan</p> <p><b>Yeast <math>\beta</math>-Glucan Amplifies Phagocyte Killing of iC3b-opsonized Tumor Cells via CR3-Syk-PI3-kinase Pathway</b></p> <p>The Journal of Immunology, 2006, Aug 1;177(3):1661-9.</p> <p>PMID: 16849475 [PubMed - indexed for MEDLINE]</p>	<p>Anti-tumor mAbs hold promise for cancer therapy, but are relatively inefficient. Therefore, there is a need for agents that might amplify the effectiveness of these mAbs. One such agent is <math>\beta</math>-glucan, a polysaccharide produced by fungi, yeast, and grains, but not mammalian cells. <math>\beta</math>-glucans are bound by C receptor 3 (CR3) and, in concert with target-associated complement fragment iC3b, elicit phagocytosis and killing of yeast. <math>\beta</math>-glucans may also promote killing of iC3b-opsonized tumor cells engendered by administration of anti-tumor mAbs. In this study, we report that tumor-bearing mice treated with a combination of <math>\beta</math>-glucan and an anti-tumor mAb show almost complete cessation of tumor growth. This activity evidently derives from a 25-kDa fragment of <math>\beta</math>-glucan released by macrophage processing of the parent polysaccharide. This fragment, but not parent <math>\beta</math>-glucan, binds to neutrophil CR3, induces CBRM 1/5 neoepitope expression, and elicits CR3-dependent cytotoxicity. These events require phosphorylation of the tyrosine kinase, Syk, and consequent PI3K activation because <math>\beta</math>-glucan-mediated CR3-dependent cytotoxicity is greatly decreased by inhibition of these signaling molecules. Thus, <math>\beta</math>-glucan enhances tumor killing through a cascade of events, including in vivo macrophage cleavage of the polysaccharide, dual CR3 ligation, and CR3-Syk-PI3K signaling. These results are important inasmuch as <math>\beta</math>-glucan, an agent without evident toxicity, may be used to amplify tumor cell killing and may open new opportunities in the immunotherapy of cancer.</p>
<p>Daniel J. Allendorf, Jun Yan, Gordon D. Ross, Richard D. Hansen, Jarek T. Baran, Krishnaprasad Subbarao, Li Wang, and Bodduluri Haribabu</p> <p><b>C5a-Mediated Leukotriene B4-Amplified Neutrophil Chemotaxis Is Essential in Tumor Immunotherapy Facilitated by Anti-Tumor Monoclonal Antibody and <math>\beta</math>-Glucan</b></p> <p>The Journal of Immunology, 2005, 174: 7050-7056.</p>	<p>Intravenous and orally administered <math>\beta</math>-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 <math>\beta</math>-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 <math>\beta</math>-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<sup>-/-</sup> 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-1<sup>-/-</sup>) mice.</p>

<p>PMID: 15905548 [PubMed - indexed for MEDLINE]</p>	
<p>Jun Yan, Daniel J Allendorf &amp; Brian Brandley</p> <p><b>Yeast Whole Glucan Particle <math>\beta</math>-Glucan in Conjunction with Antitumour Monoclonal Antibodies to Treat Cancer</b></p> <p>Expert Opinion on Biological Therapy May 2005, Vol. 5, No. 5, Pages 691-702</p> <p>PMID: 15934844 [PubMed - indexed for MEDLINE]</p>	<p>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 administered yeast <math>\beta</math>-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 <math>\beta</math>-glucan with antitumour mAb therapy in terms of tumour regression and long-term survival. It is proposed that the addition of <math>\beta</math>-glucan will further improve the clinical therapeutic efficacy of antitumour mAbs in cancer patients.</p>
<p>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</p> <p><b>Mechanism by Which Orally Administered <math>\beta</math>-1,3-Glucans Enhance the Tumoricidal Activity of Antitumor Monoclonal Antibodies in Murine Tumor Models</b></p> <p>The Journal of Immunology, 2004, 173: 797-806.</p> <p>PMID: 15240666 [PubMed - indexed for MEDLINE]</p>	<p>Antitumor mAb bind to tumors and activate complement, coating tumors with iC3b. Intravenously administered yeast <math>\beta</math>-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 <math>\beta</math>-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 <math>\beta</math>-1,3;1,6-glucan functioned similarly to barley <math>\beta</math>-1,3;1,4-glucan with antitumor mAb. With both oral <math>\beta</math>-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 <math>\beta</math>-1,3-glucan were labeled with fluorescein to track their oral uptake and processing in vivo. Orally administered <math>\beta</math>-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 <math>\beta</math>-1,3-glucans into smaller soluble <math>\beta</math>-1,3-glucan fragments that were taken up by the CR3 of marginated granulocytes. These granulocytes with CR3-bound <math>\beta</math>-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.</p>
<p>Hong, F., Hansen, R. D., Yan, J., Allendorf, D. J., Baran, J. T., Ostroff, G. R., and Ross, G. D.</p> <p><b><math>\beta</math>-Glucan Functions as an Adjuvant for Monoclonal Antibody Immunotherapy by Recruiting Tumoricidal Granulocytes as Killer Cells</b></p> <p>Cancer Research, 63(24):9023-31, Dec. 15, 2002.</p> <p>PMID: 14695221 [PubMed - indexed for Medline]</p>	<p>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. <math>\beta</math>-glucan might function as an adjuvant for antitumor mAbs. <math>\beta</math>-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 <math>\beta</math>-Glucans was limited by levels of natural antibodies and by tumor escape through elimination of antigen-positive cells. Accordingly, it was hypothesized that <math>\beta</math>-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<sub>D2</sub> ganglioside) or recombinant human MUC1. In comparison with antitumor mAb or <math>\beta</math>-glucan alone, combined treatment with mAb plus <math>\beta</math>-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. <math>\beta</math>-Glucan enhancement of the mAb tumoricidal response did not occur in mice deficient in either leukocyte CR3 (CD11b<sup>-/-</sup>) or serum C3, confirming the requirement for CR3 on leukocytes and iC3b on tumors. Granulocytes appeared to be primarily responsible for tumoricidal activity, because <math>\beta</math>-Glucan therapeutic responses did not occur in granulocyte-depleted</p>

	<p>mice. These data suggest that the therapeutic efficacy of mAbs known to activate complement (e.g. Herceptin, Rituxan and Erbitux) could be significantly enhanced if they were combined with <math>\beta</math>-Glucan.</p>
<p>Ross GD, Větvicka V, Yan J, Xia Y, Větvickova J.</p> <p><b>Therapeutic intervention with complement and beta-glucan in cancer.</b></p> <p><i>Immunopharmacology</i>. 1999 May; 42(1-3):61-74. Review.</p> <p>PMID: 10408367 [PubMed - indexed for MEDLINE]</p>	<p>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 TNF<math>\alpha</math>, IFN<math>\alpha</math>, IFN<math>\gamma</math>, and IL-6.</p>
<p>Yan, J., Větvicka, V., Xia, Y., Coxon, A., Carroll, M.C., Mayadas, T.N., and Ross, G.D.</p> <p><b><math>\beta</math>-Glucan, a "specific" biologic response modifier that uses antibodies to target tumors for recognition by complement receptor 3 (CD11b/CD18).</b></p> <p><i>J. Immunol.</i>, 163:3045-3052, 1999.</p> <p>PMID: 10477568 [PubMed - indexed for MEDLINE]</p>	<p>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.</p>

<p>Xia, Y., Větvička, V., Yan, J., Hanikýřová, M., Mayadas, T. N., and Ross, G. D.</p> <p><b>The <math>\beta</math>-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.</b></p> <p><i>J. Immunol.</i>, 162:2281-2290, 1999</p> <p>PMID: 9973505 [PubMed - indexed for MEDLINE]</p>	<p>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<sup>-/-</sup> (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 &lt;40% of normal with leukocytes from CR3<sup>-/-</sup> mice. As with neutrophils from patients with CD18 deficiency, neutrophils from CR3<sup>-/-</sup> 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<sup>-/-</sup> 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.</p>
<p>Větvička, V., Thornton, B. P., Wieman, T. J., and Ross, G. D.</p> <p><b>Targeting of NK cells to mammary carcinoma via naturally occurring tumor cell-bound iC3b and <math>\beta</math>-glucan-primed CR3 (CD11b/CD18).</b></p> <p><i>J. Immunol.</i>, 159:599-605, 1997.</p> <p>PMID: 9218574 [PubMed - indexed for MEDLINE]</p>	<p>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<sup>+</sup> 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.</p>
<p>Větvička, V., Thornton, B. P., and Ross, G. D.</p> <p><b>Soluble <math>\beta</math>-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.</b></p>	<p>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</p>

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<p><i>J. Clin. Invest.</i>, 98:50-61, 1996.</p> <p>PMID: 8690804 [PubMed - indexed for MEDLINE]</p>	<p>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.</p>
<p>Sveinbjornsson B, Olsen R, Seternes OM, Seljelid R.</p> <p><b>Macrophage cytotoxicity against murine meth A sarcoma involves nitric oxide-mediated apoptosis.</b></p> <p><i>Biochem Biophys Res Commun.</i> 1996 Jun 25;223(3):643-9.</p> <p>PMID: 8687449 [PubMed]</p>	<p>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.</p>