Orally delivered β-glucans aggravate dextran sulfate sodium (DSS)-induced intestinal inflammation

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Abstract

β-Glucans have beneficial health effects due to their immune modulatory properties. Oral administration of *β*-glucans affects tumour growth, microbial infection, sepsis, and wound healing. We hypothesized that pre-treatment with orally delivered soluble and particulate *β*-glucans could ameliorate the development of aggravate dextran sulfate sodium (DSS) induced intestinal inflammation. To study this, mice were orally pre-treated with *β*-glucans for 14 days. We tested curdlan (a particulate *β*-(1,3)-glucan), glucan phosphate (a soluble *β*-(1,3)-glucan), and zymosan (a particle made from *Saccharomyces cerevisiae*, which contains around 55% *β*-glucans). Weight loss, colon weight, and feces score did not differ between *β*-glucan and vehicle treated groups. However, histology scores indicated that *β*-glucan–treated mice had increased inflammation at a microscopic level suggesting that *β*-glucan treatment worsened intestinal inflammation. Furthermore, curdlan and zymosan treatment led to increased colonic levels of inflammatory cytokines and chemokines, compared to vehicle. Glucan phosphate treatment did not significantly affect cytokine and chemokine levels. These data suggest that particulate and soluble *β*-glucans differentially affect the intestinal immune responses. However, no significant differences in other clinical colitis scores between soluble and particulate *β*-glucans were found in this study. In summary, *β*-glucans aggravate the course of dextran sulfate sodium (DSS)-induced intestinal inflammation at the level of the mucosa.

Keywords

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1. Introduction

β-Glucans are glucose polymers consisting of a linear molecule with (1–3)-β-D-glycosidic linkages with or without side chain branches bound by (1–6)-β-D-glycoside [1]. They are major structural components of fungal cell walls and are also found in plants and some bacteria. Immune stimulation and antitumoral activities have been ascribed to β-glucans thought to be only caused by the (1,3)-β-glucans [2]. The first reported beneficial health effect of orally administered β-glucans was an antitumor effect [3] which has been studied and confirmed extensively, also in human studies [4]. In addition, oral administration of fungal β-glucans has been described to give various other health benefits during microbial infection [5,6], sepsis [7,8], radiation injury [9,10], and wound healing [11].

Unlike other glucose polymers, β-glucans are not digested upon oral administration but are taken up as they are in the small intestine. Both particulate and soluble β-glucans are absorbed by the gastrointestinal tract after which they can be found in the serum and are able to affect the immune system [5,12]. Oral administration of β-glucans has been shown to increase the number of intraepithelial lymphocytes in the intestine [13], increase TLR2 levels in the gut-associated lymphoid tissue [5], and enhance phagocytic capacity of peritoneal macrophages [12].

Dectin-1 is the main receptor for β-glucans on macrophages, dendritic cells, and neutrophils and plays an important role in anti-fungal immunity [14]. Upon β-glucan recognition dectin-1 induces various immune responses including phagocytosis, the respiratory burst, production of numerous cytokines and chemokines, and production of arachidonic acid metabolites[14]. However, recently it was shown that dectin-1 is not involved in the β-glucan–mediated protection against bacterial infection[15], and other mechanisms like the involvement of the complement system and immune system reprogramming are also thought to play a role in the immune modulatory effects of β-glucans [16,17]. Research on dectin-1–deficient mice has shown contradictory results in aggravate dextran sulfate sodium (DSS)-induced colitis models, most likely due to a difference in the microbiome composition between studies [18,19]. Hence, the role of β-glucans in mucosal immune responses is unclear.

We investigated how pre-treatment with orally delivered soluble and particulate β-glucans affects the development of DSS-induced intestinal inflammation. DSS-induced intestinal inflammation is the most widely used mouse model for human inflammatory bowel diseases (IBD). DSS causes damage to the epithelial lining of the intestine, increasing interactions of the microbiota with the intestinal immune system, which leads to an acute inflammation mainly involving innate immune cells [20]. We used curdlan, glucan phosphate, and zymosan to pre-treat mice before DSS-induced inflammation. Curdlan from the Gram-negative bacterium *Alcaligenes faecalis* is a particulate, tasteless, odorless and colorless substance that consists of solely β-(1–3)–linked glucan [21]. Glucan phosphate is isolated from *Saccharomyces cerevisiae*; like curdlan, it is tasteless, odorless, and colorless and only contains β-(1–3) glucose linkages. In contrast to curdlan, glucan phosphate is a water soluble β-glucan [22]. Zymosan is a particle made from *Saccharomyces cerevisiae* and is

often used as a fungal model. It consists of around 55% glucan, both β-(1–3)- and β-(1–6)–linked glucan, 19% mannan, 15% protein, and small amounts of fat and inorganic material [23].

We hypothesized that due to their immune modulatory role these β-glucans may positively affect the course of intestinal inflammation. The objective was to investigate the effect of oral pre-treatment, with 3 different β-glucans or β-glucan-containing preparations, in a mouse colitis model. A widely used mouse DSS colitis model, which induces an innate driven response, was used. In this study we show that pre-treatment with β-glucans is not protective and worsens intestinal inflammation in a model of DSS-induced colitis. Hence, β-glucans may contribute to the pathogenesis of innate driven acute colitis rather than improve the condition.

2. Methods and materials

2.1. Mice

C57BL/6 mice were housed and maintained under specific pathogen-free conditions in our animal facility at the Academic Medical Centre in Amsterdam. The total sample size of mice was 30, divided into 3 test groups of 8 for each group and 1 control group of 6. Animals were kept and handled in accordance with the guidelines of the Animal Research Ethics Committee of the University of Amsterdam.

2.2. Colitis experiments

Mice were sex-matched male or female and between 8 and 12 weeks of age at the time of study. Mice were pre-treated with 1 mg/0.2 mL curdlan (Sigma), glucan phosphate, or zymosan (Sigma) by oral gavage daily for 14 days before inducing DSS colitis. This dose has previously been shown to induce immune-modulating effects in mice [5]. Subsequently, 1.5% (w/v) DSS (TdB Consultancy, Uppsala, Sweden) was added to the drinking water for 6 days. Fresh DSS solutions were prepared daily. Body weights were recorded daily. After 6 days the mice were euthanized with CO_2, and organs were collected. Wet weights of the spleen and colon were recorded together with the total length of the colon. Colon weight per 6 cm was used as a disease parameter. Feces were scored as follows: (0) normal feces, (1) soft pellets, (2) thin feces,(3) watery diarrhea, and (4) bloody diarrhea [18,24].

2.3. Histology

The longitudinally divided colons were rolled, fixed in 4% formalin for 24 hours, and embedded in paraffin for routine histology [18,24]. An experienced pathologist evaluated formalin-fixed hematoxylin and eosin–stained tissue sections microscopically, in a blinded fashion. Colons were graded from 0 to 4 as an indication of incidence and severity of inflammatory lesions based on the extent of the area involved, the number of follicle aggregates, edema, fibrosis, hyperplasia, erosion/ulceration, crypt loss, and infiltration of granulocytes and mononuclear cells as indicated in the Table. The total inflammation score was calculated as the average score of the above [18,24].

2.4. Measurements of colonic cytokines

Frozen colonic tissue was homogenized in Greenberger Lysis Buffer (150 mmol/L NaCl, 15 mmol/L Tris, 1 mmol/L MgCl · $6H_2O$, 1 mmol/L $CaCl_2$, 1% Triton) with protease inhibitor cocktail from Roche (11697498001), pH 7.4, diluted 1:1 with phosphate-buffered saline, for 30 minutes on ice using a tissue homogeniser [18,25]. Protein concentrations of interleukin (IL)–12, interferon-γ (IFN-γ), tumor necrosis factor α (TNF-α), IL-10, chemokine ligand (CCL)–2, and IL-6 were measured in homogenates by cytometric bead array multiplex assay (BD Biosciences, San Jose, CA, USA) according to manufacturer protocol using flow cytometry (BD LSRFortessa). In some colon lysates too little protein was detected which made the samples unsuitable for cytokine determination; accordingly the number of samples was reduced in some groups.

2.5. Statistical analyses

The total sample size (n = 30), divided over 3 test groups (n = 8 for each group) and one control group (n = 6), was determined according to a prior statistical power analysis based on previous studies using Query Advisor, using a relevant difference in colon weight of 45 mg with a standard deviation of 10 mg in the last 6 cm of the colon with α = .05 and power = .80 [18,24,25]. All data are expressed as means ± SE. The statistical significance of the differences was evaluated using one-way analysis of variance (ANOVA) with Bonferonni posttest. Statistical significance was defined as $P < .05$.

3. Results

To determine if β-glucan feeding can reduce intestinal inflammation in DSS-induced colitis, we tested 2 different pure β-glucans and a β-glucan–containing particle, zymosan. Curdlan and glucan phosphate both consist solely of β-(1–3) glucose linkages; glucan phosphate is soluble, and curdlan is particulate. Mice were pre-treated with β-glucans by oral gavage for 14 days before inducing intestinal inflammation with DSS for 6 days (Fig. 1A). β-Glucan pre-treatment had no effect on bodyweights (data not shown). After DSS-induced colitis we found that all groups lost weight, and there was no significant difference in weight loss among the groups (Fig. 1B) nor a significant difference in colon weight (Fig. 1C). Colonic inflammation was scored by an experienced pathologist according to the Table and demonstrated that all 3 β-glucan–treated groups had a significant increase in colon pathology compared to the vehicle treated group (Figs. 1D and 2). The majority of mice had bloody diarrhea, and no significant differences in fecal score were found (Fig. 1E).

Next we investigated colonic levels of IL-12, IFN-γ, TNF-α, IL-10, CCL-2, and IL-6 in colon tissue. Levels of IFN-γ and IL-12 were below detection, as to be expected in this model [20]. TNF-α (Fig. 3A) and CCL-2 (Fig. 3B) were significantly increased in curdlan-treated mice compared to vehicle. IL-6 levels were also increased although not significantly (Fig. 3D). Zymosan-treated mice had significantly higher levels of CCL-2 and displayed a trend towards increased IL-6 compared to vehicle-treated mice (Fig. 3B and D). Unlike curdlan and zymosan, glucan phosphate–treated mice did not show significant differences with vehicle treatment in any of the measured cytokines. Interestingly, glucan phosphate–treated mice were the only group with increased IL-10 levels (Fig. 3C). Together our data

show that β-glucan feeding exacerbates intestinal inflammation in this DSS-induced colitis model.

4. Discussion

The cause of human IBD is still unclear, and current drug treatments have severe side effects and are frequently unsuccessful. Therefore, new insights on the pathogenesis of IBD and new treatment options are still required. While various health benefits are reported, the effect of β-glucans on intestinal inflammation is still uncertain. We hypothesized that due to their immune modulatory role β-glucans positively affect the course of intestinal inflammation. Therefore, we tested three β-glucan preparations that differed in origin, composition, structure, purity, and solubility. Our results however show no difference in clinical scores of DSS colitis between the β-glucan and vehicle treated groups. Interestingly, the histology score indicated that β-glucan–treated mice had increased inflammation, suggesting that β-glucan treatment worsens intestinal inflammation. Together our results suggest that both soluble and particulate β-glucans aggravate the course of DSS-induced inflammation, which refutes our hypothesis.

In our experiments we induced DSS-mediated intestinal inflammation, which is the most widely used mouse model for human colitis. In this model acute inflammation is induced by DSS, which decreases the mucus layer and disrupts the epithelial barrier [26]. Although regularly considered an acute injury rather than an IBD model, this model does demonstrate clinical and histopathological features reflecting those seen in human IBD [20]. Furthermore, a study by Melgar et al [27] shows that a variety of therapeutic agents for IBD ameliorated the inflammatory response in DSS-induced colitis with similar results to the ones reported in IBD patients. In contrast, te Velde et al [28] showed that during DSS colitis, 15 of 32 genes are transcriptionally regulated in a similar manner as in human IBD, showing the limitations of this model. Together, this indicates that DSS-induced colitis in C57BL/6 mice is relevant to human IBD pathogenesis and treatment.

Curdlan and zymosan are known to induce pro-inflammatory cytokine production by macrophages via dectin-1 signaling [29,30]. In our study, treatment with curdlan resulted in the highest inflammation score and the highest levels of colonic pro-inflammatory cytokines compared to our other groups, suggesting this β-glucan is the most potent in augmenting mucosal inflammation. Zymosan and glucan phosphate treatment gave only a slightly lower inflammation score compared to curdlan, but of these two only zymosan treated mice had increased levels of CCL-2. Particulate β-glucans are known to trigger dectin-1 signaling while soluble glucan phosphate is a dectin-1 antagonist [29,30]. However, glucan phosphate also has agonist properties as shown by its ability to activate intracellular signaling pathways, increase resistance to infection, and enhance wound repair [31–33]. The effect on the immune system may depend on the source of β-glucan since a recent study suggests that bacterial β-glucans, of which curdlan is one member, can prevent DSS colitis [34].

Our hypothesis was based on the beneficial effects seen with β-glucan treatment during microbial infection and wound healing [5,6,8,11]. In contrast, our results suggest that β-glucans aggravate intestinal inflammation in the present model. Further studies with

additional mouse strains and disease models are required to establish the generality of our observation. The increased inflammation found upon β-glucan treatment may be due to recently described training of the immune system: β-glucan treatment has been shown to enhance cytokine production upon subsequent infection, shown to be a dectin-1–mediated process [17]. Furthermore, oral glucan administration increased pattern recognition receptor expression in the gut associated lymphoid tissue of mice, and protected against subsequent infections [5]. Such a primed immune response may be useful in the fight against specific infections but detrimental during intestinal inflammation upon challenge by intestinal microbiota, in which case, a more tolerant immune system may be advantageous.

In conclusion, due to their earlier described beneficial effect during wound healing and infection we hypothesized that β-glucans could reduce intestinal inflammation. However, we found that curdlan, zymosan and glucan phosphate aggravated DSS-induced colitis, suggesting a more detrimental role for intestinal inflammation rather than a beneficial one.

Acknowledgment

REFERENCES

[1]. Williams DL, Lowman DW, Reale M, Ensley HE. Insights into the physicochemical characterization, chemistry, structure and synthesis of (13,16)-β-glucans. Chemistry and Biology of (1–3)-β-glucans; 2013 p. 29–82.

[2]. Batbayar S, Lee DH, Kim HW. Immunomodulation of fungal beta-glucan in host defense signaling by dectin-1. Biomol Ther (Seoul) 2012;20(5):433–45. [PubMed: 24009832]

[3]. Chihara G, Hamuro J, Maeda Y, Arai Y, Fukuoka F. Antitumor polysaccharide derived chemically from natural glucan (pachyman). Nature 1970;225(5236):943–4. [PubMed: 5417727]

[4]. Samuelsen AB, Schrezenmeir J, Knutsen SH. Effects of orally administered yeast-derived beta-glucans: a review. Mol Nutr Food Res 2014;58(1):183–93. [PubMed: 24019098]

[5]. Rice PJ, Adams EL, Ozment-Skelton T, Gonzalez AJ, Goldman MP, Lockhart BE, et al. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. J Pharmacol Exp Ther 2005;314(3):1079–86. [PubMed: 15976018]

[6]. Onderdonk AB, Cisneros RL, Hinkson P, Ostroff G. Anti-infective effect of poly-beta 1–6-glucotriosyl-beta 1–3-glucopyranose glucan in vivo. Infect Immun 1992;60(4):1642–7. [PubMed: 1548086]

[7]. Rasmussen LT, Fandrem J, Seljelid R. Dynamics of blood components and peritoneal fluid during treatment of murineE. coli sepsis with beta-1,3-D-polyglucose derivatives. II. Interleukin 1, tumour necrosis factor, prostaglandin E2, and leukotriene B4. Scand J Immunol 1990;32(4):333–40. [PubMed: 2173131]

[8]. Williams DL, Ha T, Li C, Kalbfleisch JH, Laffan JJ, Ferguson DA. Inhibiting early activation of tissue nuclear factor-kappa B and nuclear factor interleukin 6 with (1->3)-beta-D-glucan increases long-term survival in polymicrobial sepsis. Surgery 1999;126(1):54–65. [PubMed: 10418593]

[9]. Gu YH, Takagi Y, Nakamura T, Hasegawa T, Suzuki I, Oshima M, et al. Enhancement of radioprotection and anti-tumor immunity by yeast-derived beta-glucan in mice. J Med Food 2005;8(2):154–8. [PubMed: 16117606]

[10]. Patchen ML, MacVittie TJ. Comparative effects of soluble and particulate glucans on survival in irradiated mice. J Biol Response Mod 1986;5(1):45–60. [PubMed: 3958754]

[11]. Delatte SJ, Evans J, Hebra A, Adamson W, Othersen HB, Tagge EP. Effectiveness of beta-glucan collagen for treatment of partial-thickness burns in children. J Pediatr Surg 2001;36(1):113–8. [PubMed: 11150448]

[12]. Hunter KW, Jr, Gault RA, Berner MD. Preparation of microparticulate beta-glucan from Saccharomyces cerevisiae for use in immune potentiation. Lett Appl Microbiol 2002; 35(4):267–71. [PubMed: 12358685]

[13]. Tsukada C, Yokoyama H, Miyaji C, Ishimoto Y, Kawamura H, Abo T. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of beta-glucan. Cell Immunol 2003;221(1):1–5. [PubMed: 12742376]

[14]. Drummond RA, Brown GD. The role of dectin-1 in the host defence against fungal infections. Curr Opin Microbiol 2011; 14(4):392–9. [PubMed: 21803640]

[15]. Marakalala MJ, Williams DL, Hoving JC, Engstad R, Netea MG, Brown GD. Dectin-1 plays a redundant role in the immunomodulatory activities of beta-glucan-rich ligands in vivo. Microbes Infect 2013;15(6–7):511–5. [PubMed: 23518266]

[16]. Boxx GM, Kozel TR, Nishiya CT, Zhang MX. Influence of mannan and glucan on complement activation and C3 binding by Candida albicans. Infect Immun 2010;78(3):1250–9. [PubMed: 20028806]

[17]. Quintin J, Saeed S, Martens JH, Giamarellos-Bourboulis EJ, Ifrim DC, Logie C, et al. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. Cell Host Microbe 2012;12(2):223–32. [PubMed: 22901542]

[18]. Heinsbroek SE, Oei A, Roelofs JJ, Dhawan S, te Velde AA, Gordon S, et al. Genetic deletion of dectin-1 does not affect the course of murine experimental colitis. BMC Gastroenterol 2012;12:33–42. [PubMed: 22507600]

[19]. Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, Strom SP, et al. Interactions between commensal fungi and the C-type lectin receptor dectin-1 influence colitis. Science 2012; 336(6086):1314–7. [PubMed: 22674328]

[20]. Wirtz S, Neurath MF. Mouse models of inflammatory bowel disease. Adv Drug Deliv Rev 2007;59(11):1073–83. [PubMed: 17825455]

[21]. McIntosh M, Stone BA, Stanisich VA. Curdlan and other bacterial (1-> 3)-beta-D-glucans. Appl Microbiol Biotechnol 2005;68(2):163–73. [PubMed: 15818477]

[22]. Williams DL, McNamee RB, Jones EL, Pretus HA, Ensley HE, Browder IW, et al. A method for the solubilization of a (1–3)-beta-D-glucan isolated from Saccharomyces cerevisiae. Carbohydr Res 1991;219:203–13. [PubMed: 1804535]

[23]. Di Carlo FJ, Fiore JV. On the composition of zymosan. Science 1958;127(3301):756–7.

[24]. ten HT, Drillenburg P, Wijnholds J, Te Velde AA, Van Deventer SJ. Differential susceptibility of multidrug resistance protein-1 deficient mice to DSS and TNBS-induced colitis. Dig Dis Sci 2002; 47(9):2056–63. [PubMed: 12353855]

[25]. ten HT, Corbaz A, Amitai H, Aloni S, Belzer I, Graber P, et al. Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice. Gastroenterology 2001;121(6):1372–9. [PubMed: 11729116]

[26]. Petersson J, Schreiber O, Hansson GC, Gendler SJ, Velcich A, Lundberg JO, et al. Importance and regulation of the colonic mucus barrier in a mouse model of colitis. Am J Physiol Gastrointest Liver Physiol 2011;300(2):G327–33. [PubMed: 21109593]

[27]. Melgar S, Karlsson L, Rehnstrom E, Karlsson A, Utkovic H, Jansson L, et al. Validation of murine dextran sulfate sodium-induced colitis using four therapeutic agents for human inflammatory bowel disease. Int Immunopharmacol 2008;8(6):836–44. [PubMed: 18442787]

[28]. Te Velde AA, de KF, Sterrenburg E, Pronk I, ten Kate FJ, Hommes DW, et al. Comparative analysis of colonic gene expression of three experimental colitis models mimicking inflammatory bowel disease. Inflamm Bowel Dis 2007;13(3): 325–30. [PubMed: 17206675]

[29]. Brown GD, Gordon S. Immune recognition. A new receptor for beta-glucans. Nature 2001;413(6851):36–7.

[30]. Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of beta-glucans. J Exp Med 2003;197(9):1119–24. [PubMed: 12719478]

[31]. Luhm J, Langenkamp U, Hensel J, Frohn C, Brand JM, Hennig H, et al. Beta-(1– > 3)-D-glucan modulates DNA bindingof nuclear factors kappaB, AT and IL-6 leading to an anti-inflammatory shift of the IL-1beta/IL-1 receptor antagonist ratio. BMC Immunol 2006;7:5–19. [PubMed: 16553947]

[32]. Lyuksutova OI, Murphey ED, Toliver-Kinsky TE, Lin CY, Cui W, Williams DL, et al. Glucan phosphate treatmentattenuates burn-induced inflammation and improves resistance to Pseudomonas aeruginosa burn wound infection. Shock 2005;23(3):224–32. [PubMed: 15718919]

[33]. Li C, Ha T, Kelley J, Gao X, Qiu Y, Kao RL, et al. Modulating Toll-like receptor mediated signaling by (1->3)-beta-D-glucan rapidly induces cardioprotection. Cardiovasc Res 2004;61(3): 538–47. [PubMed: 14962484]

[34]. Lee KH, Park M, Ji KY, Lee HY, Jang JH, Yoon IJ, et al. Bacterial beta-(1,3)-glucan prevents DSS-induced IBD by restoring the reduced population of regulatory T cells. Immunobiology 2014;219(10):802–12. [PubMed: 25092569]
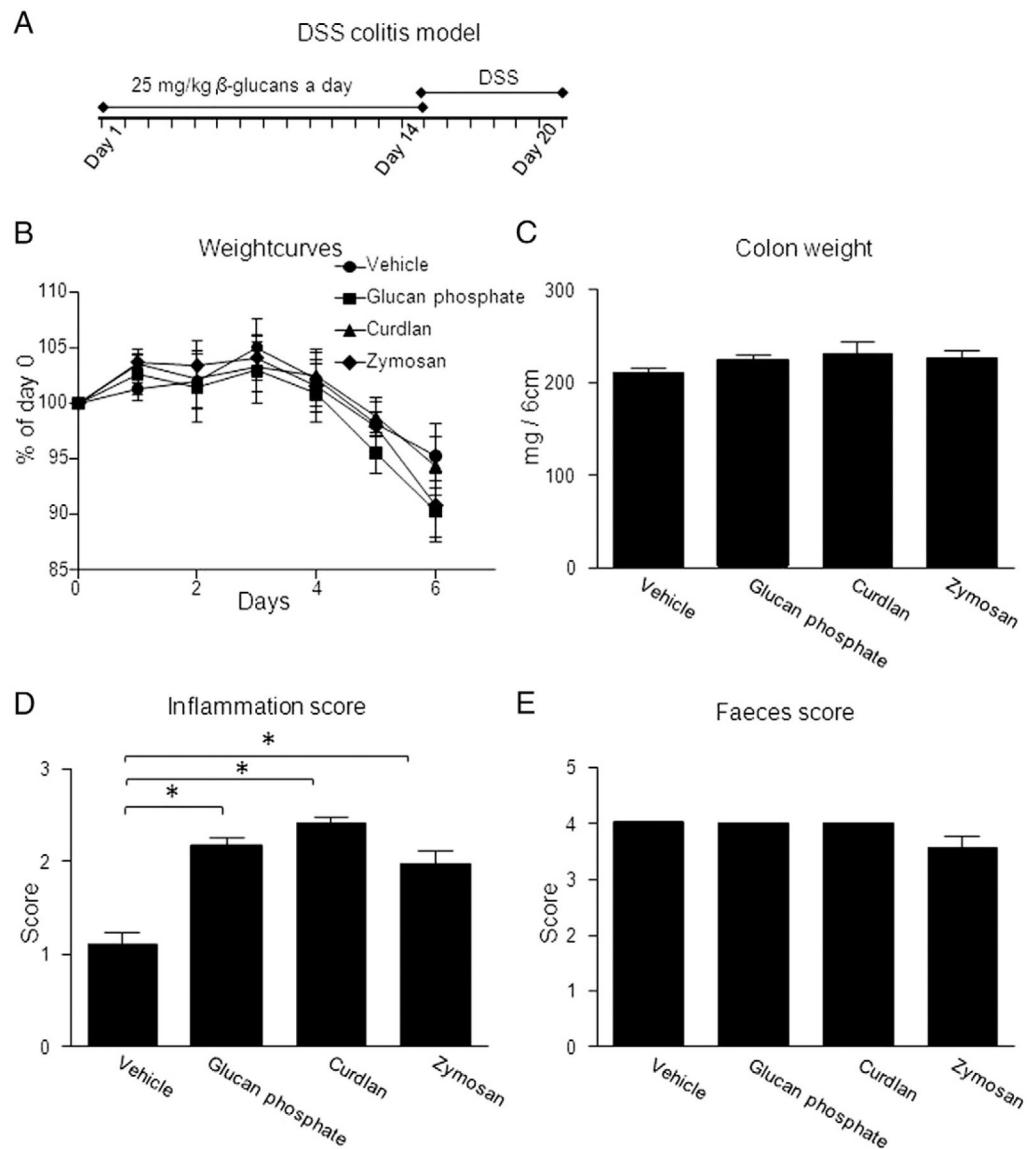
Fig. 1 - .

β-Glucan pre-treatment in DSS-induced intestinal inflammation. A, Mice were given vehicle (n = 6), glucan phosphate (n = 8), curdlan (n = 8) or zymosan (n = 8) once daily by oral gavage for two weeks before inducing DSS colitis with 1.5% (w/v) DSS in drinking water. The mice were euthanized after 6 days of DSS treatment. B, Body weights were measured as indication of disease severity in this model; weights are shown as percentage of weight compared to those on day 1. C, Colons were weighed as a measure of inflammation, shown as weight per 6 cm colon. D, Colon pathology scores were determined by an experienced pathologist. Colons were graded from 0 to 4 points as indicated in Table. E, Fecal scores were given ranging from 0 to 4 points. The values represent the means ± SE. One-way ANOVA with bonferonni post-test was used for statistical analysis. *P < .05.
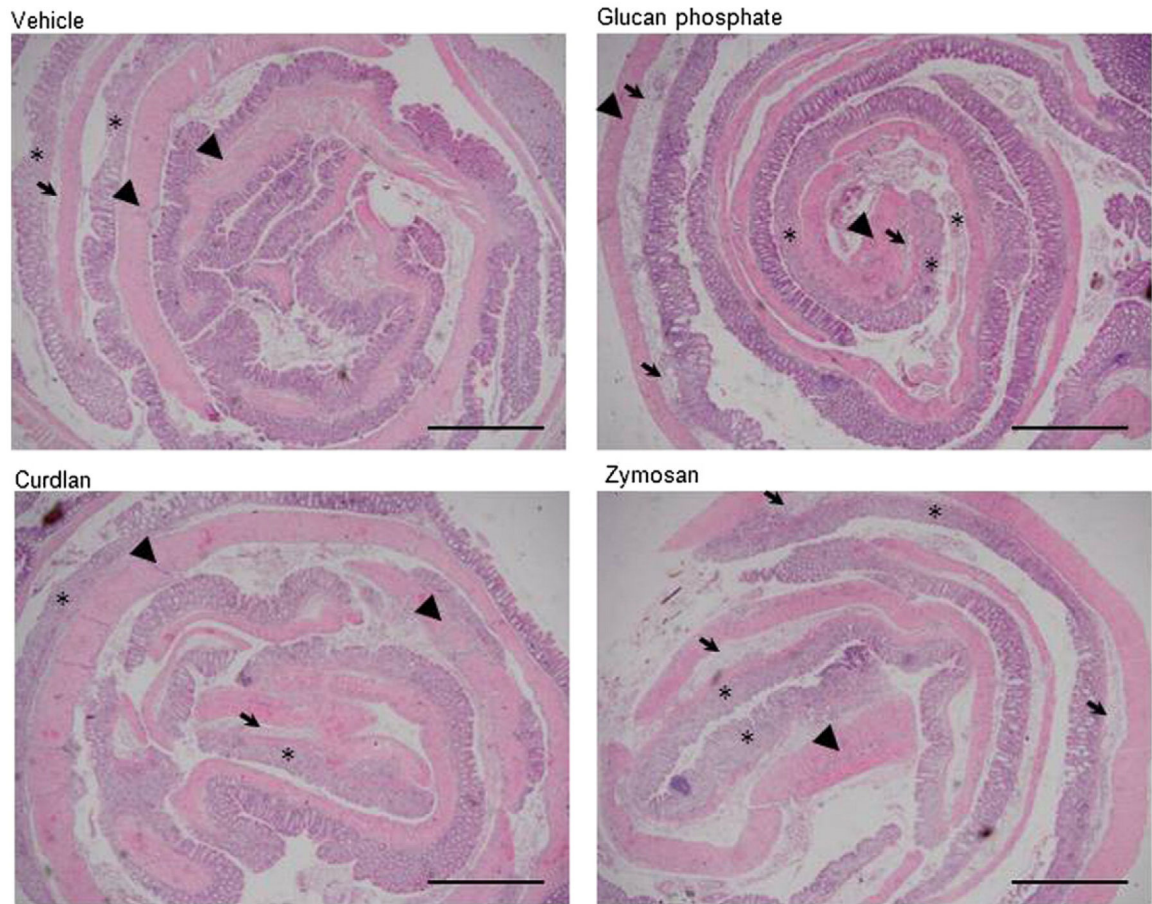
Fig. 2 - .

Colon histology of β-glucan pre-treatment in DSS-induced intestinal inflammation. Representative picture of the colon sections stained with hematoxylin and eosin. In inflamed areas crypt loss occurred (asterisk), accompanied by a large amount of cell infiltration in the mucosa. Cell infiltration frequently extended to the submucosa (arrows) and occasionally to the muscularis externa (arrowheads). Bar, 200 μm.
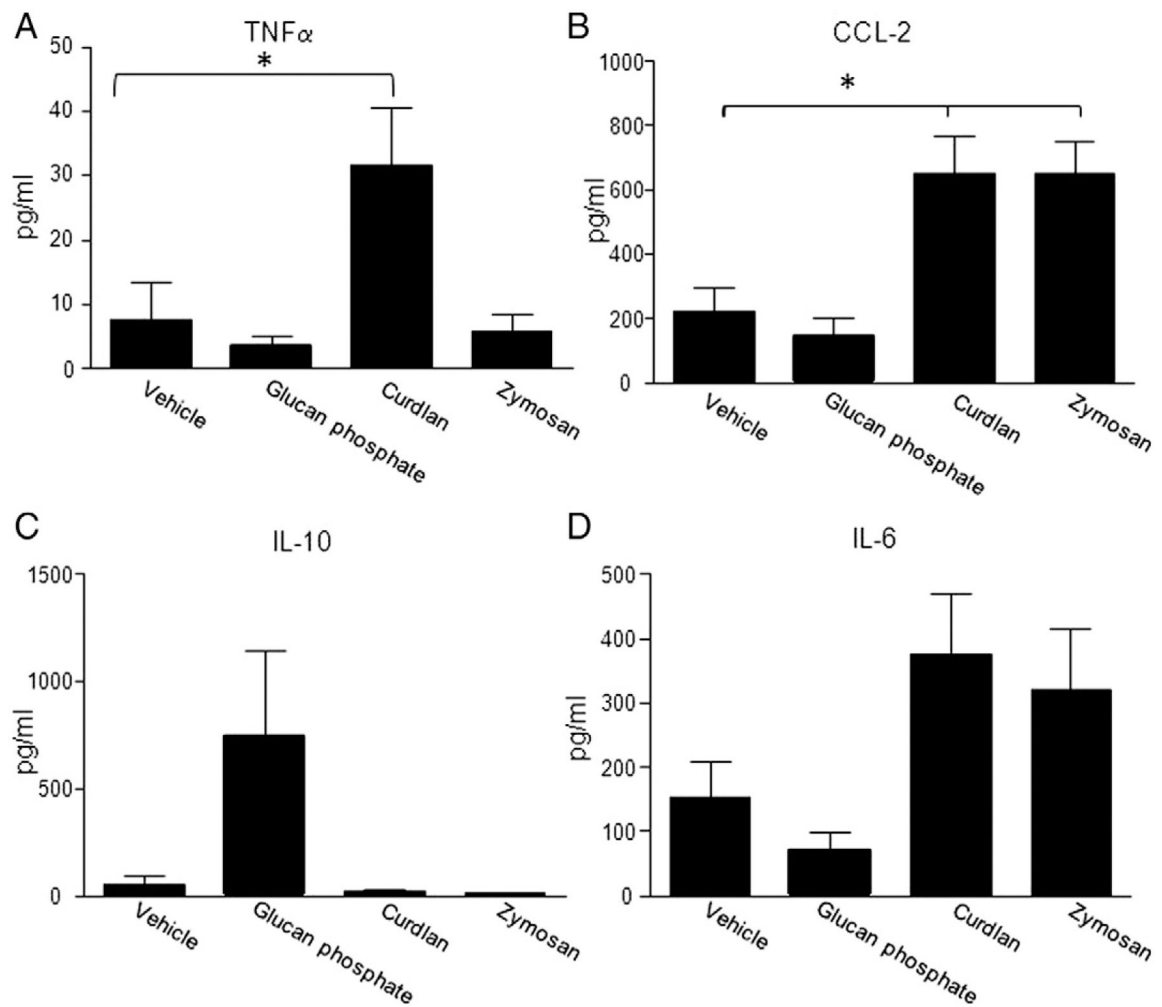
Fig. 3 - .

Inflammatory cytokines levels in colon. Mice were given vehicle, glucan phosphate, curdlan or zymosan once daily for two weeks before inducing DSS colitis with 1.5% (w/v) DSS in drinking water. Cytokine protein concentrations were determined in colon lysates, vehicle (n = 4), glucan phosphate (n = 6), curdlan (n = 5) or zymosan (n = 8). A, TNF-α concentrations. B, CCL-2 concentrations. C, IL-10 concentrations. D, IL-6 concentrations. The sample means are indicated with a line. One-way ANOVA with bonferonni post-test was used for statistical analysis. *P < .05.

Table-

Colitis total inflammation score

score	0	1	2	3	4
Area involved	0%	1%–10%	10%–25%	25%–50%	>50%
Follicles	Normal (0–1)	Minimal (2–3)	Mild (4–5)	Moderate (6–7)	Severe (>7)
Edema	Absent	Minimal	Mild	Moderate	Severe
Fibrosis	Absent	Minimal	Mild	Moderate	Severe
Erosion/ulceration	0%	1%–10%	10%–25%	25%–50%	>50%
Crypt loss	0%	1%–10%	10%–25%	25%–50%	>50%
Granulocytes	Normal	Minimal increase	Mild increase	Moderate increase	Severe increase
Mononuclear cells	Normal	Minimal increase	Mild increase	Moderate increase	Severe increase

The total inflammation score was determined by the average score of the following criteria: area involved, the number of follicle aggregates, edema, fibrosis, hyperplasia, erosion/ulceration, crypt loss and infiltration of granulocytes and mononuclear cells.