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Original Research

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

Sigrid E.M. Heinsbroek[a,*], *David L. Williams*[b], *Olaf Welting*[a], *Sybren L. Meijer*[c], *Siamon Gordon*[d], *Wouter J. de Jonge*[a]

[a] *Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, University of Amsterdam, AMC, Amsterdam, The Netherlands*
[b] *Department of Surgery, James H. Quillen College of Medicine and Centre of Excellence in Inflammation, Infectious Disease and Immunity, East Tennessee State University, Johnson City, TN, USA*
[c] *Department of Pathology, Academic Medical Centre, University of Amsterdam, AMC, Amsterdam, The Netherlands*
[d] *Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*

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ABSTRACT

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

1. Introduction

ß-Glucans are glucose polymers consisting of a linear molecule with (1-3)-ß-D-glycosidic linkages with or without side chain branches bound by (1-6)-ß-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 ß-glucans thought to be only

* *Corresponding author at:* Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, Meibergdreef 69–71, 1105BK Amsterdam, The Netherlands. Tel.: +31 20 5668060.
E-mail address: s.e.heinsbroek@amc.nl (S.E.M. Heinsbroek).

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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 $MgCl \cdot 6H_2O$, 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