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Immunomodulatory and therapeutic potential of a mucin-specific mycelial lectin from *Aspergillus panamensis*

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ABSTRACT

The present study reports the evaluation of immunomodulatory and therapeutic potential of a purified *Aspergillus panamensis* lectin. The immunomodulatory potential of the purified lectin was determined in swiss albino mice by studying its effect on anaphylaxis reaction, arthus reaction, respiratory burst activity, nitric oxide production and quantification of cytokine levels. The therapeutic potential of the lectin was evaluated in male wistar rat models by studying its curative effect on ulcerative colitis. The purified lectin inhibited systemic anaphylaxis and arthus reaction. It enhanced the functional ability of macrophages which was evident from increase in reduction of nitroblue tetrazolium dye and nitric oxide production. It also stimulated the production of Th-1 cytokine IFN-γ and Th-2 cytokine IL-6. Maximum immunomodulatory effect was seen at lectin concentration of 1.5 mg/kg body weight. The lectin also showed curative effect against trinitrobenzene sulphonic acid induced ulcerative colitis. The results of this study adequately reflect the role of purified *A. panamensis* lectin in improving the immune status of mice models. They also show the effect of lectin in reducing the severity of incidence and decrease in clinical symptoms of ulcerative colitis.

1. Introduction

Lectins are proteins or glycoproteins possessing at least one non-catalytic domain that binds reversibly to a specific mono- or oligosaccharide resulting in agglutination of cells or precipitation of glycoconjugates [1]. Glycans present on cell membranes mediate cell-cell and cell-matrix environment interactions. Extrinsic effects such as modulation of cellular function can be seen due to lectin-glycan interactions [2]. Lectins from animals and plants have been thoroughly investigated, but comparatively lesser information is available on fungal lectins. Lectins from mushrooms have been characterized for their medical and therapeutic potential, but microfungal lectins have not been extensively explored for these activities so far [3]. Mushroom lectins exhibit a wide range of biological activities like antitumor activity, hypotensive activity, immunomodulatory activity, HIV-reverse transcriptase inhibitory activity, mitogenic activity etc [4]. Immune system is the first barrier in the prevention of diseases and hence immunomodulators are key components in modern health and wellness industries. The natural immunomodulators act to strengthen weak immune system and to moderate overly active immune system. Over the past few years, their market share has increased rapidly due to wide ranging medical applications for stimulation and suppression of the immune system [5].

Inflammatory bowel disease (IBD) is a destructive, chronic, inflammatory disorder caused due to a dysregulated mucosal immune response to intestinal microbacteria in genetically susceptible host [6]. Ulcerative colitis (UC) and Crohn's disease (CD) are two types of inflammatory bowel diseases having highest prevalence in the world population. The possible mechanisms by which ulcerative colitis is induced include persistant specific infection, abnormal ratio of beneficial and detrimental commensal microbial agents (dysbiosis), defective mucosal barrier function, defective microbial clearance and aberrant immunoregulation [7]. Currently drugs used for the treatment of inflammatory bowel disease include aminosalicylates, corticosteroids and biological drugs such as anti-cytokine drugs and anti-cell adhesion molecules. Advances in treatment for IBD include biological therapies based on monoclonal antibodies or fusion proteins [8]. Current research is focused on better understanding of immunologic pathways causing IBD and developing targeted therapies [7]. Nevertheless, it has been reported that only one third patients achieve sustained remission

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resulting in an urgent need for new therapies for treatment of inflammatory bowel disease [9].

In a previous study by our group, the purification and characterization of an intracellular lectin isolated from *Aspergillus panamensis* has been reported. The lectin was also found to exhibit mitogenic activity towards mouse splenocytes and antibacterial activity against Gram positive and Gram negative bacteria [10]. The objective of the present study was to evaluate the immunomodulatory property of purified *Aspergillus panamensis* lectin. Its therapeutic effect on the immune mediated disease ulcerative colitis has also been evaluated.

2. Materials & methods

2.1. Extraction and purification of Aspergillus panamensis lectin

The fungal culture was procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India and was grown and maintained as previously described [10]. Briefly, the culture was cultivated in Erlenmeyer's flasks (1.0 l) containing 500.0 ml medium by inoculating 5 agar discs covered with mycelium (10 mm diameter) and incubating under stationary conditions at 30 °C for 7 days. The lectin was purified by single step affinity chromatography and then subjected to SDS-PAGE analysis as described previously [10]. Briefly, fungal mycelia were homogenized followed by grinding with acidified sand in phosphate buffered saline (PBS, 0.1 M, pH 7.2) containing 1 mM benzamidine hydrochloride and centrifuged at 3000 × *g* for 20 min at 4 °C. The supernatant was loaded on porcine stomach mucin coupled sepharose 4 B affinity column and eluted with 1.0 M urea. Lectin positive fractions were pooled and dialysed against PBS (0.1 M, pH 7.2) and their lectin activity and protein content was analyzed. Purity of the lectin was evaluated by using SDS-PAGE with coomassie brilliant blue staining. Purified *A. panamensis* lectin with specific activity of 32.0 mg/ml was used for further experiments.

2.2. Hemagglutination assay

Human blood type O erythrocytes were used to assess the hemagglutination activity as described earlier [11].

2.3. Animals

Male swiss albino mice (20–25 g) were procured from Institute of Microbial Technology, Chandigarh, India. Male rats from wistar strain (200–220 g) were procured from Animal House, Guru Jambeshwar University of Science and Technology, Hisar, Haryana. The animals were housed in our animal facility under controlled temperature (25 ± 2 °C) with 12 h light/dark cycle and fed standard pellet diet (Kissan Feeds Ltd., Mumbai, India). Handling of animals was in accordance with the guidelines of CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests, Government of India. Experimental protocols were in compliance with Institutional Animal Ethics Committee, Punjabi University, Patiala (Permit No. 107/99/CPCSEA/2014).

2.4. Immunomodulatory activity

The immunomodulatory potential of purified *A. panamensis* lectin was determined in male swiss albino mice by evaluating its effect on systemic anaphylaxis reaction, arthus reaction and immunomodulatory parameters such as respiratory burst activity, nitric oxide production and quantification of cytokines interleukin-6 (IL-6) and interferon-γ (IFN-γ). Levamisole was used as the standard immunomodulator at the concentration of 2.5 mg/kg body weight based on previous studies [12,13].

2.4.1. Systemic anaphylaxis reaction

Systemic anaphylaxis reaction was evaluated according to the method described previously [14]. The animals were divided into five groups (n = 5). Group I (positive control) animals were treated with PBS (0.1 M, pH 7.2) on days −6, −3, 0, 3, 6, 9 and 12 and then sensitized by intraperitoneal administration of 1 mg bovine serum albumin (BSA) in 0.2 ml aluminium hydroxide suspension (15 mg/ml) on day 0. The animals were shocked intravenously by 1 mg BSA in 0.2 ml PBS (0.1 M, pH 7.2) on day 17. Group II (negative control) animals were treated with PBS (0.1 M, pH 7.2) on days −6, −3, 0, 3, 6, 9 and 12 and then sensitized by intraperitoneal administration of 1 mg BSA in 0.2 ml almunium hydroxide suspension (15 mg/ml) on day 0. The animals were shocked by 1 mg ovalbumin in 0.2 ml PBS (0.1 M, pH 7.2) on day 17. Group III (levamisole treated) animals were treated with levamisole (2.5 mg/kg body weight) on days −6, −3, 0, 3, 6, 9 and 12 and then sensitized by intraperitoneal administration of 1 mg BSA in 0.2 ml aluminium hydroxide suspension (15 mg/ml) on day 0. The animals were shocked by 1 mg BSA in 0.2 ml PBS on day 17. Group IV (test group) animals were treated with levamisole (2.5 mg/kg body weight) on days −6, −3, 0, 3, 6, 9 and 12. They were sensitized by intraperitoneal administration of 1 mg BSA in 0.2 ml aluminium hydroxide suspension (15 mg/ml) on day 0. Animals were shocked by intravenous administration of BSA (1 mg in 0.2 ml PBS, 0.1 M, pH 7.2) after 1 h of intraperitoneal administration of 150 μg of purified lectin. Group V (lectin control) animals were treated with PBS (0.1 M, pH 7.2) on days −6, −3, 0, 3, 6, 9 and 12. They were sensitized by intraperitoneal injection of 1.5 mg lectin/kg body weight on day 0. Animals were shocked by intravenous administration of 1.5 mg lectin/kg body weight in 0.2 ml PBS (0.1 M, pH 7.2) on day 17. Systemic anaphylaxis reaction was observed within 30 min after the shocking injection. Death or inactivity of the mice was considered as a positive reaction, while reaction was considered negative, when no changes in the behavior of mice were observed and their movement remained normal.

2.4.2. Arthus reaction

Arthus reaction was assessed in mice as described previously [14]. Mice were divided into six groups (n = 6). Group I (untreated control) animals were kept on normal diet and were not given any treatment throughout the experiment. Group II (immunized control) animals were intraperitoneally administered 0.2 ml PBS (0.1 M, pH 7.2) on days −6, −3, 0, 3, 6, 9 and 12. Group III (levamisole treated) animals were intraperitoneally administered 2.5 mg/kg body weight of levamisole on days −6, −3, 0, 3, 6, 9 and 12. Group IV (test group) animals were intraperitoneally administered 1.5 mg of purified lectin/kg body weight on days −6, −3, 0, 3, 6, 9 and 12. Group V (test group) animals were intraperitoneally administered 3.0 mg of purified lectin/kg body weight on days −6, −3, 0, 3, 6, 9 and 12. Group VI (test group) animals were intraperitoneally administered 4.5 mg of purified lectin/kg body weight on days −6, −3, 0, 3, 6, 9 and 12. On the 14th day, 20 μl of BSA (0.5 mg/ml) was injected intradermally into the left footpad of mice in all the groups and an equal amount of PBS (0.1 M, pH 7.2) was injected into the right footpad as a control. The thickness of footpads was measured using a micro-caliper at 0, 2, 24, 48 and 72 h after injection of BSA and PBS. The difference in the thickness of left and right footpad was calculated and taken as a measure of arthus reaction. Results are expressed as mean ± standard deviation (S.D.).

2.4.3. Other immunological parameters

The other immunological parameters assessed were respiratory burst activity, nitric oxide production and quantification of cytokines. The animals were divided into six groups (n = 6). Group I