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White button, portabella, and shiitake mushroom supplementation up-regulates interleukin-23 secretion in acute dextran sodium sulfate colitis C57BL/6 mice and murine macrophage J.744.1 cell line

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ABSTRACT

Interleukin-23 (IL-23), a cytokine produced primarily by dendritic cells, is involved in host defense against gut pathogens and promotes innate immunity and inflammatory responses through the IL-23/interleukin-17 axis. We previously reported that extracts from edible mushrooms enhanced antimicrobial α-defensin production n HL60 cells. Because IL-23 is involved in defensin production, we hypothesized that edible mushrooms may modulate its secretion and gut inflammation. Eight-week-old C57BL/6 mice were fed the AIN76 diet or the same diet supplemented with 5% white button (WBM), portabella, or shiitake mushrooms. To assess in vivo and in vitro cytokine secretion, 7 to 8 mice per group received 3% dextran sodium sulfate (DSS) in drinking water during the last 5 days of the 6-week feeding period. To delineate the mechanisms by which mushrooms alter IL-23 secretion, J.744.1 cells were incubated with (100 μg/mL) WBM, portabella, and shiitake extracts without and with 100 μg/mL curdlan (a dectin-1 agonist) or 1 mg/mL laminarin (a dectin-1 antagonist). The dectin-1 receptor is a pattern-recognition receptor found in phagocytes, and its activation promotes antimicrobial innate immunity and inflammatory responses. In DSS-untreated mice, mushrooms significantly increased IL-23 plasma levels but decreased those of interleukin-6 (IL-6) ($P < .05$). In DSS-treated mice, mushroom-supplemented diets increased IL-6 and IL-23 levels ($P < .05$). Mushroom extracts potentiated curdlan-induced IL-23 secretion, and mushroom-induced IL-23 secretion was not blocked by laminarin in vitro, suggesting the involvement of both dectin-1–dependent and dectin-1–independent pathways. Although all mushrooms tended to increase IL-6 in the colon, only WBM and shiitake tended to increase IL-23 levels. These data suggest that edible mushrooms may enhance gut immunity through IL-23.

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Abbreviations: DSS, Dextran sodium sulfate; GI, Gastrointestinal; IL-6, Interleukin-6; IL-17, Interleukin-17; IL-23, Interleukin-23; MPO, Myeloperoxidase; PM, Portabella mushroom; SM, Shiitake mushroom; WBM, White button mushroom.

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1. Introduction

The gastrointestinal (GI) tract is a vital organ in nutrient absorption and host defense against pathogens [1,2]. Approximately 90% of pathogens enter into the body through the mucosa [1]. The GI immune system is composed of mesenteric lymph nodes, Peyer's patches, and lamina propria–associated lymphocytes [3]. Unlike other peripheral immune organs, the GI immune system has the constant challenge of responding to pathogens while promoting tolerance to food antigens and commensal microflora [3].

Cytokines produced by the intestinal dendritic cells play a crucial role in modulating innate and adaptive immunity [4]. Although several cytokines are involved in mucosal defense against pathogens, interleukin-23 (IL-23) is of specific interest because of its capacity to regulate the immune response through 3 different pathways [5,7]. First, in conjunction with interleukin-6 (IL-6) and transforming growth factor β, IL-23 stimulates naïve $CD4^+$ T cells to differentiate into a novel subset of cells called Th17 cells [8]. These Th17 cells secrete a cytokine called IL-17, which promotes neutrophil recruitment at sites of inflammation [9,10]. Second, the IL-23/IL-17 axis plays a major role in local intestinal inflammation and production of defensins in gut mucosa [11,12] and lungs [13], which is another route of pathogen entry. Defensins are very well known for their high potency to lyse gram-positive and gram-negative bacteria, viruses, and fungi [14]. Third, IL-23 promotes immunological memory by stimulating $CD4^+$ T memory cell proliferation [15], which suggests that increased mucosal IL-23 production may amplify or improve mucosal immunity against various pathogens [16].

The immunomodulatory and antimicrobial properties of certain edible mushrooms, such as, shiitake mushroom (SM), have been previously established, but their effects on IL-23 secretion have not yet been characterized. Previously, we reported that the extracts from edible mushroom enhance α-defensin production in the human promyelocytic cell line HL60 [17]. Because IL-23/IL-17 axis is involved in the production of certain types of defensins (β-defensin-2) in gut mucosa [11,13], we hypothesized that these edible mushrooms may enhance acute gut innate immune and inflammatory responses through IL-23 induction in vivo. Although immunostimulatory properties of β-glucan via dectin-1 receptor expressed by macrophages and monocytes have been reported [18,19], there are virtually no data on the immunomodulatory effect of white button mushrooms (WBMs) and portabella mushrooms (PMs) through IL-23 pathways.

Therefore, the goal of the current study is to investigate the effects of mushroom supplementation of the AIN76 diet on inflammatory, immune, and cytokine responses in dextran sodium sulfate (DSS)–induced acute colitis C57BL/6 mice. We used DSS-induced acute colitis models instead of chronic colitis models to focus on acute inflammatory responses such as increased neutrophilic infiltration and function as well as IL-6 and IL-23 cytokine responses. Because macrophages and dendritic cells express dectin-1 receptor and secrete IL-23 cytokine, we used mouse J744A.1 monocytic cell line to study mechanisms by which mushrooms or mushroom extracts modulate IL-23 secretion. Moreover, dectin-1 receptor signal activation leads to increased antimicrobial innate immunity and inflammatory responses.

2. Methods and materials

2.1. Materials

Materials and supplies were purchased from the following vendors: Mouse cell line J744A.1 from American Type Culture Collection (Manassas, VA); DSS from MP Biomedicals (Solon, OH); RPMI-1640 and medium supplements, sodium pyruvate, L-glutamine, nonessential amino acids, fetal calf serum, Dulbecco's Modified Eagle Medium from Invitrogen (Grand Island Biological Company, Grand Island, NY); curdlan (from *Alcaligenes daecalis*), laminarin (from *Laminaria digitala*); lipopolysaccharide (from *Escherichia coli*) from Sigma Chemical Company (St Louis, MO); AIN76; and the same diet supplemented with WBM, PM, and SM from Harlan Teklad (Indianapolis, IN). Cytokine assay kits (IL-6 and IL-23) were purchased from R & D Systems (Minneapolis, MN) and MPO from Hycult Biotech (Hycult Biotech, Frontstraat 2a, 5405 PB Uden, The Netherlands). Mushrooms were a gift from J&M Mushrooms (Miami, OK).

2.2. Mice

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Oklahoma State University (IACUC no. HE-072). Eight-week-old female C57BL/6 mice (n = 62) were purchased from Jackson Laboratories (Bar Harbor, ME) and acclimatized to our laboratory conditions for 7 days while being fed the AIN76 diet. After the acclimation period, mice were fed either the AIN76-control diet or the same diet supplemented with 5% WBM, PM, or SM (n = 14-16 per group) for 6 weeks. The AIN76 provides all macronutrients (proteins, fat, and carbohydrates), minerals, trace elements, and vitamins to the maintenance requirement of laboratory rodents [20]. Mice had access to their feed and water 24 h/d. Environmental conditions of the animal facility were set at 22°C and 12 hours, light to dark cycle. To assess the effects of baseline and induced in vivo cytokine secretion, 7 to 8 mice in each dietary treatment group received 3% DSS in drinking water during the last 5 days of the 6-week feeding period. This is a standard protocol used in studies of gut immunity and inflammation in rodents [21].

2.3. Organ collection and histopathology

Following DSS administration, mice were anesthetized by CO_2 inhalation for 60 seconds and weighed. Blood samples were collected from retro-orbital plexus in sodium heparin–containing tubes. After allowing samples to stand at room temperature for 60 minutes, they were centrifuged at 400*g* at room temperature for 10 minutes. Plasma was collected and immediately frozen at –80°C until used for cytokine measurement. Thymuses were also removed and weighed to assess the effect of dietary and DSS treatments.