Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.nrjournal.com

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

Lawrance C. Chandra[a,*], *Djibril Traoré*[a], *Christine French*[a], *Denver Marlow*[b,c], *Jean D'Offay*[b], *Stephen L. Clarke*[a], *Brenda J. Smith*[a], *Solo Kuvibidila*[a,d]

[a] *Department of Nutritional Sciences, College of Human Sciences, Oklahoma State University (OSU), Stillwater, OK 74078, USA*
[b] *Department of Physiological Sciences & Veterinary Pathobiology, Center for Veterinary Health Sciences (CVHS), OSU, Stillwater, OK 74078, USA*
[c] *Department of Comparative Medicine, Kansas State University, Manhattan, KS 66506, USA*
[d] *The Research Institute for Children's Hospital, Louisiana State University Health Sciences Center, Department of Pediatrics, 200 Henry Clay, New Orleans, LA 70118, USA*

ARTICLE INFO

Article history:
Received 6 July 2012
Revised 13 February 2013
Accepted 20 February 2013

Keywords:
IL-6
IL-23
Edible mushrooms
Gut immunity
Mice
Curdlan
Laminarin

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.

Published by Elsevier Inc.

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.

* *Corresponding author*. Division of Comparative Pathology, Diplomate, American College of Laboratory Animal Medicine, Tulane National Primate Research Center, Covington, LA 70433-8915, USA. Tel.: +1 985 871 6561; fax: +1 985 871 6510.
E-mail address: lchandra@tulane.edu (L.C. Chandra).

0271-5317/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.nutres.2013.02.009

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.

To determine the effects of mushrooms on gut anatomy/morphology following induction of inflammation by DSS, mice were euthanized, and colons were removed. We measured the total length of the colon, which is an indicator of colonic inflammation or colitis [22]. A small piece (0.5 cm) from the distal part of the colon was placed in 10% buffered formalin for histopathologic analysis, and the remaining portion of colon was frozen at –80°C until used for colon homogenate preparation. Formalin-fixed colon tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for further histologic evaluation. The severity of colon inflammation and the presence of the number of lymphoid follicles were evaluated in a blinded manner. The inflammation score was based on the extent of infiltration of inflammatory cells (most of which are neutrophils), mucosal injury, loss of crypts, and surface epithelium on a scale of 0 to 4, as described in the literature [23].

2.4. *Preparation of colon homogenates for measurement of cytokines and myeloperoxidase activity*

The remaining portion of colons were homogenized in 1-mL ice-cold lysis buffer (radio-immunoprecipitation assay buffer) containing 1% (vol/vol) of a protease inhibitor cocktail and 1% (vol/vol) of a phosphatase inhibitor cocktail (Cell Signaling Technology, Inc, Danvers, MA) by using a tissue homogenizer (VWR PowerMax Advanced Homogenizing System 200 987556). The lysate was centrifuged at 14000*g*, 4°C for 15 minutes, and the supernatant was transferred to 1.7-mL microfuge tubes and immediately frozen at –80°C until used for cytokines and myeloperoxidase assays [24].

2.5. *Culture of J744A.1 cells*

To begin to determine the potential mechanisms through which mushroom extracts modulate cytokine secretion, we used the mouse J744A.1 monocytic cell line, which can be differentiated into cells with a dendritic cell phenotype and are a major source of IL-23 [25]. J744A.1 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 100-mL fetal calf serum/L, 2 mmol/L glutamine, 100 U/L penicillin and 100 mg/L streptomycin, 1.5 mg/mL $NaHCO_3$ and 50 μmol/L β-mercaptoethanol at 37°C, and 5% CO_2 in a humidified incubator [25]. Cell viability was assessed by trypan blue dye exclusion method. For cytokine secretion, 5 $\times 10^5$ viable cells were seeded into 6-well tissue culture plates (2 mL per well) and incubated with lipopolysaccharide (100 ng/mL), WBM (100 μg/mL), PM (100 μg/mL), SM (100 μg/mL), alone or in combination with curdlan (100 μg/mL) or with laminarin (1 mg/mL) for 3 and 6 days. Curdlan [26] and laminarin [27] are dectin-1 receptor agonist and antagonist, respectively [16]. At the end of the incubation period, plates were centrifuged at 400*g*, 4°C for 10 minutes. Supernatants were collected, aliquoted (1000 μL per vial), and frozen at –80°C until used for cytokine assays.

2.6. *Cytokine measurement*

Interleukin-23 and IL-6 were measured in plasma, culture supernatants, or colon homogenates by enzyme linked immunosorbent assay kits (R&D System, Minneapolis, MN, USA) [28,29]. Myeloperoxidase (MPO) in colon homogenates was also measured by using murine MPO enzyme linked immunosorbent assay kit (Hycult Biotechnology) [30].

2.7. *Statistical analyses*

Results are expressed as means ± SEM. Data analyses were performed using SPSS 17 or Microstatistical program. Post hoc multiple comparisons (Tukey test) were made whenever F values obtained after analysis of variance reached significance. Multiple regression analysis was also performed to determine the measurements that most explained the variance in gut immunity assessed by colon inflammatory scores. Correlation coefficients between cytokines and other measurements were also calculated. $P \leq .05$ was considered significant. The sample size was sufficient to detect a 20% difference at 80% power in plasma levels of inflammatory cytokines.

3. Results

3.1. *Effect of dietary treatment on body and thymus weights*

At the beginning of the feeding period, the time of DSS administration (0 day of DSS administration) and 5 days post-DSS administration (time of euthanization), there were no significant differences in mean body weights among the 4 study groups (Table 1). However, at the time of euthanization, all mice, irrespective of the dietary interventions, showed significant body weight losses as compared with the time before DSS ($P < .05$). Specifically, within the same dietary treatment group, mean final body weights of mice that received DSS were significantly lower than those of DSS-untreated mice that were fed the same diet ($P < .05$). We observed no significant difference in absolute and relative mean thymus weights by dietary treatment, and none of the mushroom-supplemented diets prevented thymus atrophy associated with DSS administration (Table 1).

3.2. *Effect of mushroom supplementation on plasma IL-23 with or without DSS administration*

In DSS-untreated mice, despite the unexpected biological variation, a PM-supplemented diet significantly increased IL-23 plasma levels compared with mice fed the control (AIN76) diet ($P < .05$; Fig. 1A). Interleukin-23 was undetectable in the plasma of mice fed SM-supplemented diet. In DSS-treated mice, all the mushroom-supplemented diets significantly increased plasma IL-23 levels above those found in DSS-untreated mice (Fig. 1B; $P < .05$). Among dietary treatments, mice fed the WBM-supplemented diet had the highest plasma IL-23 levels, followed by mice fed the PM and SM-supplemented diets. The differences were statistically significant (Fig. 1B. $P < .05$).

3.3. *Effect of mushroom supplementation on plasma IL-6 levels in DSS-treated and DSS-untreated mice*

In DSS-untreated mice, WBM, PM, and SM decreased plasma IL-6 levels by 55%, 78%, and 93%, respectively, compared with

Table 1 – The weights of body and lymphoid organs and food intake as a function of dietary and DSS treatments

	AIN76	WBM	PM	SM
No DSS				
Wt-0, g	19.25 ± 1.25	18.32 ± 0.63	19.47 ± 0.48	19.33 ± 0.71
Wt-final, g	21.64 ± 0.51 *	21.62 ± 0.73 †	21.93 ± 0.25 ‡	19.95 ± 0.69 §
Thymus, mg	51.14 ± 6.04 *	53.09 ± 9.68 †	65.23 ± 6.52 ‡	48.36 ± 7.19 §
Thymus, mg/g Wt	2.37 ± 0.29 *	2.40 ± 1.09 †	2.99 ± 0.33 ‡	2.39 ± 0.29 §
Food intake, g/d	4.82 ± 0.35	4.88 ± 0.32	4.86 ± 0.28	4.78 ± 0.33
With DSS				
Wt-0, g	18.74 ± 1.06	18.60 ± 1.07	18.71 ± 0.77	18.93 ± 0.61
Wt-DSS, g	21.56 ± 1.3	21.78 ± 0.98	21.59 ± 1.32	21.28 ± 0.94
Wt-final, g	17.57 ± 0.53 ‖	16.43 ± 0.87 ‖	17.77 ± 0.71 ‖	16.62 ± 0.38 ‖
Thymus, mg	27.53 ± 5.56	27.00 ± 3.43	29.83 ± 3.79	24.31 ± 3.14
Thymus, mg/g Wt	1.53 ± 0.27	1.54 ± 0.17	1.65 ± 0.17	1.45 ± 0.17
Food intake, g/d before DSS	4.76 ± 0.28	4.83 ± 0.31	4.85 ± 0.38	4.71 ± 0.28

Values are expressed as means ± SEM; n = 7 to 8. Abbreviations: AIN76, control diet (American Institute for Nutrition); Wt, weight.
* Within each dietary treatment and with a specific measurement (ie, thymus), means of DSS-untreated mice with control diet are significantly different from those of DSS-treated mice ($P < .05$).
† Within each dietary treatment and with a specific measurement (ie, thymus), means of DSS-untreated mice with WBM are significantly different from those of DSS-treated mice ($P < .05$).
‡ Within each dietary treatment and with a specific measurement (ie, thymus), means of DSS-untreated mice with PM are significantly different from those of DSS-treated mice ($P < .05$).
§ Within each dietary treatment and with a specific measurement (ie, thymus), means of DSS-untreated mice with SM are significantly different from those of DSS-treated mice ($P < .05$).
‖ $P < .05$, mean body weights of mice fed the same diet were significantly lower after DSS than before DSS.

those fed the control diet, and the decrease was statistically significant ($P < .05$) (Fig. 1C). In DSS-treated mice, dietary treatment significantly increased IL-6 plasma levels by 2.38-, 3.65-, and 1.41-fold in mice fed WBM-, PM-, and SM-supplemented diets, respectively, compared with those fed the control diet ($P < .05$). Within the same dietary treatment,

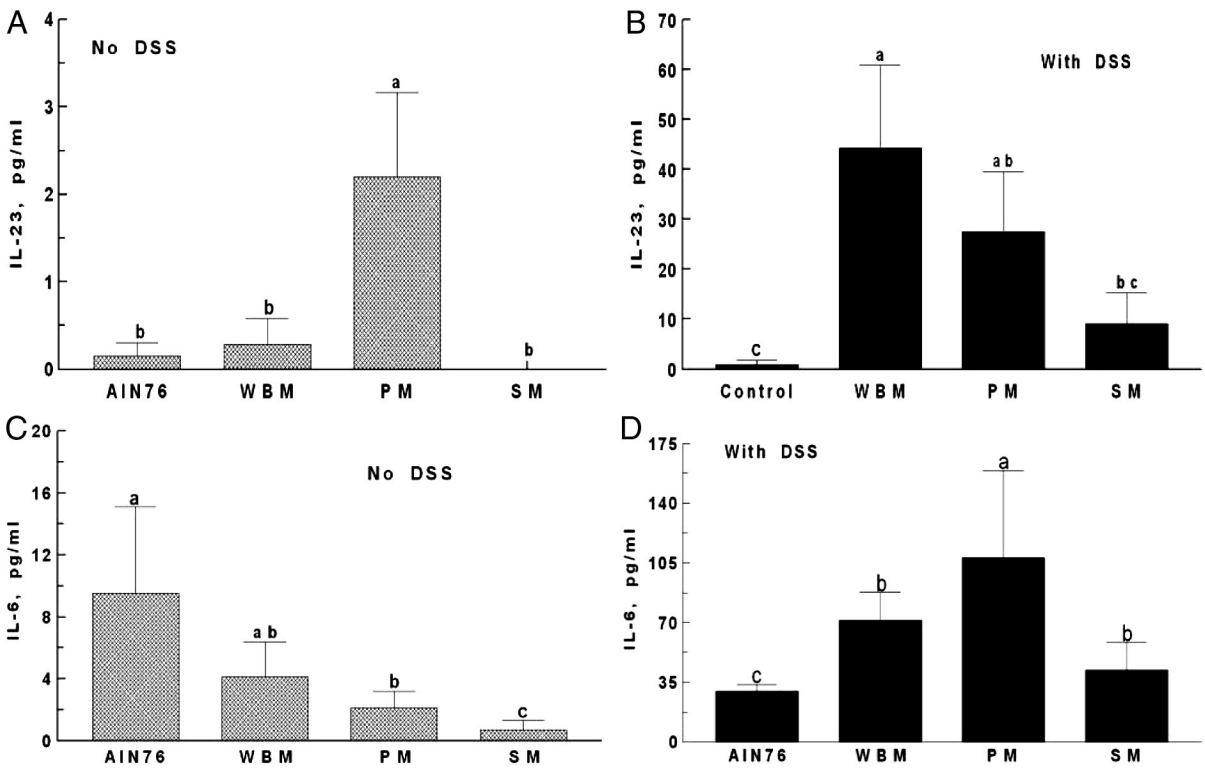

Fig. 1 – Effect of different dietary treatment on plasma IL-23 and IL-6. Abbreviation: AIN76, baseline diet (American Institute for Nutrition). Values are means ± SEM; n = 7 to 8 per group. Bars with unlike superscript letters are significantly different: a > b > c; $P < .05$.

Table 2 – Inflammatory markers in colon homogenates as a function of dietary treatment and DSS

	AIN76	WBM	PM	SM
No DSS				
IL-6, pg/mg protein	1.05 ± 0.59[c]	2.34 ± 0.41[b]	1.38 ± 0.29[c]	3.93 ± 1.35[a §]
IL-23, pg/mg protein	1.66 ± 1.12	3.55 ± 0.70	1.10 ± 0.55	1.94 ± 0.73 [§]
Colon length, cm	6.30 ± 0.17 [*]	6.46 ± 0.15 [†]	6.5 ± 0.12 [‡]	6.64 ± 0.24 [§]
Lymphoid follicles/10 fields	4.80 ± 1.19[b]	4.00 ± 2.03[b]	7.40 ± 1.57[a]	3.75 ± 1.11[c]
MPO U/mg protein	34.73 ± 17.79	21.4 ± 32.88 [†]	13.45 ± 4.44 [‡]	24.77 ± 5.47 [§]
With DSS				
IL-6, pg/mg protein	1.41± 0.42	7.44 ± 4.29	1.37 ± 0.72	0.96 ± 0.16
IL-23, pg/mg protein	0.77 ± 0.47	1.85 ± 1.08	1.37 ± 0.96	0.29 ± 0.15 [‖]
Colon length, cm	4.89 ± 0.29	4.41 ± 0.21	4.88 ± 0.49	4.79 ± 0.16
Lymphoid follicles/10 fields	6.86 ± 1.54[a]	4.38 ± 1.10[a]	3.00 ± 0.88[c]	3.29 ± 0.61[b]
MPO U/mg protein	51.22 ± 11.68	64.7 ± 11.88	58.03 ± 6.02	45.71 ± 4.74

Values are expressed as means ± SEM; n = 7 to 8. Abbreviations are as defined in Table 1. Means with different superscript letters are significantly different: a > b > c; $P < .05$.

[*] Within each column or dietary treatment and with a specific measurement, means of DSS-untreated mice with for control diet are significantly different from those of DSS-treated mice ($P < .05$).

[†] Within each column or dietary treatment and with a specific measurement, means of DSS-untreated mice with WBM are significantly different from those of DSS-treated mice ($P < .05$).

[‡] Within each column or dietary treatment and with a specific measurement, means of DSS-untreated mice with PM are significantly different from those of DSS-treated mice ($P < .05$).

[§] Within each column or dietary treatment and with a specific measurement, means of DSS-untreated mice with SM are significantly different from those of DSS-treated mice ($P < .05$).

[‖] $P = .1$, SM compared with AIN76.

administration of DSS significantly increased IL-6 plasma levels by 3.9- to 51.4-fold.

3.4. *Effect of mushroom supplementation on colon cytokine levels*

In DSS-untreated mice, the levels of IL-6 (pg/mg protein) in the colon were very low, and the values ranged from 0 to 9.27 pg/mg protein. Supplementation of the control diet with 5% of lyophilized mushrooms tended to increase mean IL-6 in the colon (analysis of variance, $P = .092$). When Student t tests were performed to compare SM vs control diets, SM increased colon IL-6 levels above those observed in mice fed the control diet by a factor of 3.75 (Table 2; $P < .05$). White button mushroom and PM also exhibited the same trend, although a smaller stimulatory effect on IL-6 than SM (medians, pg/mg protein: 0.745, 2.5, 1.37, and 2.65 for control, WBM-, PM-, and SM-supplemented diets, respectively). Dextran sodium sulfate administration increased the mean IL-6 levels in mice fed the WBM-supplemented by about 3-fold, although the increase was not statistically significant because of wide intra-group variation. In mice fed the SM-supplemented diet, colon IL-6 levels significantly decreased after DSS administration ($P < .05$; Table 2).

Although the differences did not reach statistical significance, in DSS-untreated mice, WBM and SM elevated the IL-23 in the colon by 113.9% and 16.9%, respectively (Table 2). In contrast to WBM and SM, PM reduced the mean levels by 33.7%, although this reduction was not statistically significant. Dextran sodium sulfate treatment also did not significantly alter colon IL-23 levels. However, SM-supplemented diet significantly reduced mean colon IL-23 in DSS-treated vs DSS-untreated mice ($P < .05$) and also tended to decrease the means in respect to mice fed the control diet ($P = .1$).

3.5. *Effect of mushroom supplementation on colon length and lymphoid follicles*

Mushrooms did not significantly affect colon length in DSS-treated and DSS-untreated mice (Table 2). However, regardless of dietary treatment, the mean colon length was significantly reduced by 22% to 32% after DSS treatment (Table 2; $P < .05$). In DSS-untreated mice, PM was the only mushroom that significantly increased the mean lymphoid follicle numbers ($P < .05$; Table 2). In DSS-treated mice, all mushrooms decreased the mean lymphoid follicle numbers relative to the AIN76 diet ($P < .05$). Within the same dietary treatment groups, DSS treatment reduced the mean lymphoid follicle numbers in mice fed mushroom-supplemented diets but not in those fed the control diet (Table 2; $P < .05$).

3.6. *Effect of mushroom supplementation on colon MPO*

In DSS-untreated mice, mushrooms tended to decrease the mean MPO, a measure of neutrophil infiltration in the colon when adjusted to milligrams per protein relative to mice fed the control diet. However, the decrease was not significant (Table 2). In DSS-treated mice, mushrooms also did not significantly affect MPO levels. However, within the same dietary treatment, as expected, DSS significantly increased MPO levels ($P < .05$).

3.7. *Effect of mushroom treatment on colon histology*

As expected after DSS treatment, all mice, irrespective of their dietary treatments, developed inflammation and infiltration of neutrophils and mononuclear cells, which are evidenced by colon histopathology and high inflammation scores (Fig. 2). After DSS treatment, only mice fed the control diet

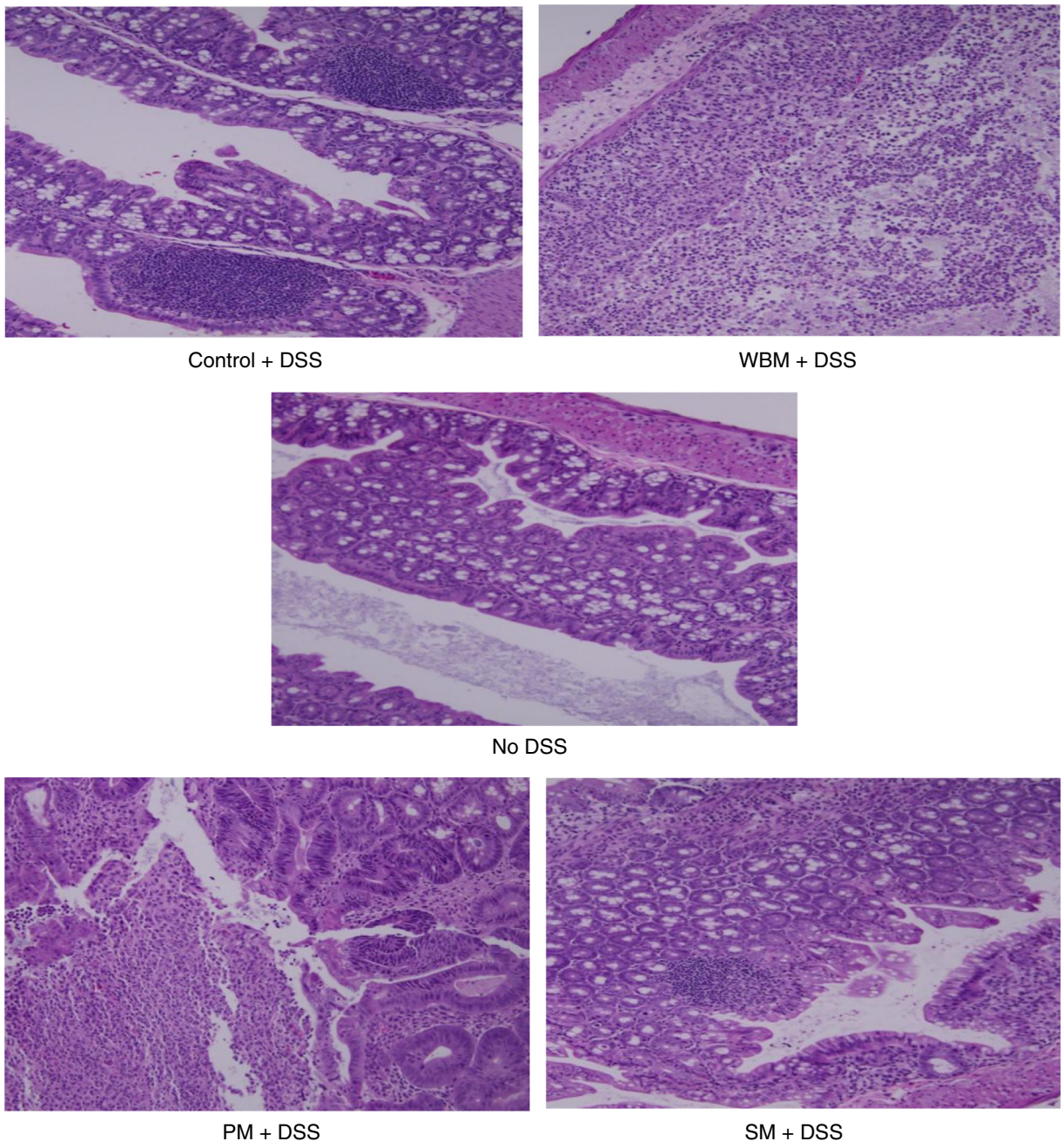

Fig. 2 – Effect of mushroom supplementation on colon histology. Abbreviation: AIN76, baseline diet (American Institute for Nutrition). Abbreviation: WBM, white button mushrooms; PM, portabella mushrooms; SM, shitake mushrooms; DSS, dextran sodium sulfate.

exhibited hyperplasia lymphoid follicles (Fig. 2) compared with those fed mushroom-supplemented diets. Mean inflammatory scores varied from 2 to 4 in DSS-treated mice vs 0.6 or less in DSS-untreated mice (Fig. 3; $P < .05$). Differences in inflammatory scores varied as follows: WBM > PM > SM (Fig. 3; $P < .05$).

3.8. *Effect of mushroom extracts on in vitro IL-23 secretion by J744A.1 cells*

Except for PM, IL-23 in the supernatant of J744A.1 cell cultures treated for 3 days with various compounds were undetectable (data not shown). After the sixth day of cell activation, IL-23 was detectable in the supernatant of control (baseline), WBM, PM, WBM + curdlan, PM + curdlan, and SM + curdlan cultures (Fig. 4). Interestingly, WBM and SM, but not PM, extracts augmented the curdlan-induced IL-23 secretion (Fig. 4; $P < .05$). However, unexpectedly, mushroom extracts induced by IL-23 secretion were not blocked by laminarin (Fig. 4).

3.9. *Multiple regression analysis and correlation coefficients*

When all DSS-treated mice were pooled, multiple regression analysis suggested that plasma IL-6, plasma IL-23, and

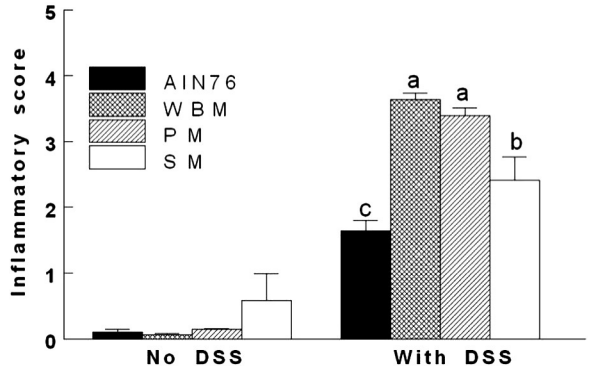

Fig. 3 – Effect of mushroom supplementation on colon inflammatory scores. Abbreviation: AIN76, baseline diet (American Institute for Nutrition). Values are means ± SEM; n = 7 to 8 per group. Bars with unlike superscript letters are significantly different: a > b > c; P < .05.

number of colon follicles explained 36.29%, 24.39%, and 26.43% of the variance of gut inflammatory scores, respectively ($P < .05$). When all mice were pooled, DSS treatment, plasma IL-6, plasma IL-23, and colon follicle numbers explained 54.57%, 29.53%, 16.67%, and 10.49% of the variance of gut inflammation, respectively ($P < .05$). Diet, colon IL-6, colon IL-23, and MPO only explained between 2.11% and 4.02% of variance of gut inflammation. The inflammatory scores of the colon positively and significantly correlated with MPO, plasma IL-6, and plasma IL-23; and colon IL-6 positively correlated with colon IL-23, MPO and plasma IL-23 and plasma IL-6 ($P < .05$).

4. Discussion

Inflammation is the complex biological response of vascularized living tissues of the body against any harmful stimuli.

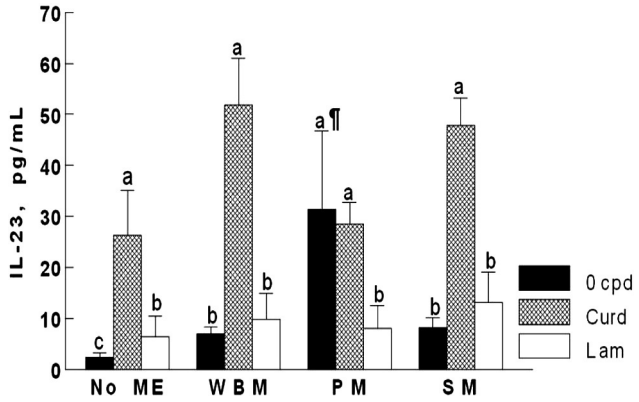

Fig. 4 – Effect of mushroom extracts on in vitro IL-23 secretion by J744A.1 cells. Abbreviation: ME, mushroom extracts. Values are means ± SEM, n = 3 to 4 experiments. Within the same mushroom treatment group, bars with unlike superscript letters are significantly different: a > b > c, P < .05. ¶P < .05, PM baseline IL-23 level compared with WBM and SM baseline.

Inflammation can be broadly classified into 2 categories, namely, acute and chronic inflammation. Acute inflammation is an immediate immune mediated defensive response for any noxius stimuli, whereas chronic inflammation is an unwanted inflammatory response leading to many chronic diseases [31]. In this study, we used 3% DSS oral administration to investigate the immunomodulatory effects of edible mushrooms on acute inflammation. The DSS-induced acute colitis model is the accepted model to study innate immunity, acute inflammation, and neutrophilic function [22]. Interestingly, in healthy mice (not treated with DSS), 5% PM mushroom supplementation significantly increased the plasma IL-23 concentrations. Although all mushroom supplementation caused significant increases in IL-23 plasma levels in DSS-treated mice, there were differences in IL-23 response among mushroom diets. White button mushroom had the greatest immunomodulatory effects, whereas SM had the least effect. This is in agreement with our earlier findings in which different mushroom showed species specific response on arthritis and liver fat content in mice [29,32].

Based on the results of our in vitro study and in vivo study with DSS-treated mice, it appears that WBM (and PM) may have different immune response in normal healthy individual subjects vs patients. In individuals with infection or prone for more infection such as those patients being treated for cancer, under immunosuppressive therapy, WBM (and PM) may be beneficial because they would stimulate acute inflammatory and immune responses [33]. Because 90% of pathogens get into the body through mucosa, by up-regulating IL-23 secretion, the risk of chronic inflammation may be limited due to the rapid clearance of infectious agents through mucosal entry. However, in patients with autoimmune diseases where exaggerated immune response is directed to the self-antigens, the frequent consumption of high amounts of WBM and PM may not be beneficial because of the possible risk of overproduction of IL-23. However, as of now, we do not have any data to suggest that consumption of 1 regular serving of WBM per day would induce acute and/or chronic inflammation in humans.

Because macrophage/dendritic cells are the major source of IL-23 production, we used J744A.1, a mouse monocytic cell line to determine the mechanism by which (WBM and PM) mushrooms up-regulate IL-23 production. We observed that the addition of mushroom extracts to J744A.1 cell cultures induced the secretion of IL-23 on day 6, suggesting that the beneficial effects of mushrooms may be time dependent. However, interestingly, only PM extracts up-regulated IL-23 secretion on day 3 of cultures, which suggests that PM is more potent in up-regulating IL-23 compared with WBM and SM. Based on our experimental findings, it is evident that PM may up-regulate IL-23 levels in both in vivo and in vitro models.

White button mushroom and SM extract potentiated curdlan effect on IL-23 secretion. Curdlan is a dectin-1 receptor agonist known to induce the secretion of various cytokines, including IL-23 [26]. The additive effect of mushroom extracts and curdlan suggests that mushrooms potentiate IL-23 secretion through dectin-1 receptor. However, mushroom-induced IL-23 secretion was not blocked by laminarin, a dectin-1 receptor antagonist, suggesting a dectin-1 receptor independent pathway is also involved in mushroom-induced IL-23

production. In contrast to WBM and SM, PM extracts did not potentiate a curdlan effect on IL-23 secretion, suggesting a dectin-1 receptor independent pathway.

The reason for the conflicting results may be related to the differences in the β-glucan structures. The structure of *Agaricus bisporus* (WBM and PM) β-glucan is α-1,6 [1,4] glucan compared with α-1,3 [1,6] for *Lentinula edodes* (SM) [34,35]. Mushrooms are rich in β-glucan, which is the natural ligand for dectin-1 receptor present in the macrophages and dendritic cells [18,19]. In a recent study, curdlan, an isolated β-glucan from *Sparassis crispa* was shown to increase the secretion of IL-23, IL-6, and interleukin-1β by human dendritic cells isolated from healthy humans [16]. Moreover, the curdlan-stimulated dendritic cells helped to differentiate naive CD4 cells into Th17 [16]. Thus, the increase in IL-23 secretion by WBM and PM in the mouse cell line is in agreement with the observation made with primary human dendritic cells.

In our study, mushroom dietary supplementation also increased IL-6 concentrations in plasma of DSS-treated mice. This observation is possibly expected as an attempt to down-regulate the secretion of the proinflammatory cytokine—IL-23 when the mucosal barrier is compromised. Interleukin-23, in conjunction with IL-6, interleukin-1β, and transforming growth factor β, stimulates naive $CD4^+$ T cells to differentiate into a novel subset of cells called Th17 cells, which secretes IL-17 [36,37]. This IL-17 cytokine is involved in the recruitment of neutrophils, the first line of body defense against bacterial pathogens to the site of inflammation [10].

After DSS treatment, all mice, irrespective of their dietary treatments, developed inflammation and infiltration of neutrophils and mononuclear cells, which is evidenced by colon histopathology and high inflammation scores. Among dietary treatments, WBM had high infiltration of neutrophils and mononuclear cells with a high mean inflammation score, followed by PM, SM, and control. In addition, the WBM-treated mice had an elevated colonic MPO level, which is the biomarker for neutrophilic and acute inflammatory response. Moreover, neutrophils are also required for wound healing [38]. However, we are unable to document that mushroom-induced acute inflammatory and cytokine responses that helped in a rapid mucosal healing process and bacterial clearance because we euthanized all mice during the peak stage of acute inflammation. Nevertheless, these results suggest that edible mushroom dietary supplementation might shift the immune response toward Th17 paradigm through IL-23/IL-17 axis and promote acute inflammatory responses, which is beneficial in the pathogen killing and wound healing processes. We would like to state that excess proinflammatory and unresolved inflammatory responses may not be good for autoimmune and chronic diseases.

Interestingly, mice fed the control diet showed hyperplasia of lymphoid follicles after DSS treatment. Lymphoid hyperplasia, a sign of chronic inflammation, is frequently found in the histopathology of colonic tumors [39]. This colonic histologic finding suggests that mushroom supplementation promotes acute inflammatory response, which is beneficial during infection, as opposed to chronic inflammation, which may increase the risk of malignancies. Furthermore, our study supports previous research findings in which fish oil with or without curcumin increased acute inflammatory responses in the DSS colitis mouse model [40].

In summary, the new and most important findings of the current study are the following: (*a*) in DSS-untreated mice, PM increased the plasma IL-23, whereas SM blocked it and WBM had no effect; (*b*) in DSS-treated mice, all mushrooms up-regulated the plasma IL-23 levels; (*c*) although all mushrooms induced inflammation in DSS-treated mice, WBM and PM had the most potentiating properties; and (*d*) WBM, PM, and SM stimulate IL-23 production through both dectin-1–dependent and dectin-1–independent mechanisms. To the best of our knowledge, this is the first report showing that edible mushrooms such as WBM and PM increase innate and gut inflammatory responses and up-regulate IL-23 levels in both in vivo and in vitro models. Health benefits and human nutrition application of this study include using WBM and PM to increase innate immunity against pathogens and immunological memory against vaccines. Some of the limitations of our study include lack of measurement of IL-17, challenging mushroom diet–fed animal with gut pathogens to see the translational health benefits. These experiments are the focus of our next study.

Acknowledgment

We thank USDA (Mushroom Council Grant no. 580790706), Oklahoma State University startup funds, and OCAST (Oklahoma Center for Advancement of Science and Technology Grant no. HR09-147S) for providing financial support for this study. We also thank Jim & M mushrooms for their generosity in supplying mushrooms for our study. The authors would like to thank Anita Pere for her assistance in proof reading of this manuscript.

REFERENCES

[1] Nagler-Anderson C. Man the barrier! Strategic defences in the intestinal mucosa. Nat Rev Immunol 2001;1:59–67.
[2] Singh V, Singh K, Amdekar S, Singh DD, Tripathi P, Sharma GL. Innate and specific gut-associated immunity and microbial interference. FEM (Fed Eur Microbiol Soc) 2009;55:6–12.
[3] Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol 2003;3(4):331–41.
[4] Perdigón G, Maldonado Galdeano C, Valdez JC, Medici M. Interaction of lactic acid bacteria with the gut immune system. Eur J Clin Nutr 2002;56(Suppl 4):S21–6.
[5] Doisne JM, Soulard V, Bécourt C, Amniai L, Henrot P, Havenar-Daughton C, et al. Cutting edge: crucial role of IL-1 and IL-23 in the innate IL-17 response of peripheral lymph node NK1.1-invariant NKT cells to bacteria. J Immunol 2011;186(2):662–6.
[6] Brereton CF, Sutton CE, Ross PJ, Iwakura Y, Pizza M, Rappuoli R, et al. Escherichia coli heat-labile enterotoxin promotes protective Th17 responses against infection by driving innate IL-1 and IL-23 production. J Immunol 2011;183:5896–906.
[7] Ciccia F, Bombardieri M, Principato A, Giardina A, Tripodo C, Porcasi R, et al. Overexpression of interleukin-23, but not interleukin-17, as an immunologic signature of subclinical intestinal inflammation in ankylosing spondylitis. Arthritis Rheum 2009;60(4):955–65.

[8] Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem 2003;278(3):1910–4.
[9] Maloy KJ, Kullberg MC. IL-23 and Th17 cytokines in intestinal homeostasis. Mucosal Immunol 2008;5:339–49.
[10] McAleer JP, Kolls JK. Mechanisms controlling Th17 cytokine expression and host defense. J Leukoc Biol 2011;90:263–70.
[11] Monteeone I, Pallone F, Monteleone G. Interleukin-23 and Th17 cells in the control of gut inflammation. Article OD297645Mediat Inflamm 2009:1–7, http://dx.doi.org/10.1155/2009/297645.
[12] Uhlig HH, McKenzie BS, Hue S, Thompson C, Joyce-Shaikh B, Stepankova R, et al. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. Immunity 2006;25(2):309–18.
[13] Kao CY, Chen Y, Thai P, Wach S, Huang F, Kim C, et al. IL-17 markedly up-regulates β-defensin-2 expression in human airway epithelium via JAK and NF-κB signaling pathways. J Immunol 2004;173:3482–91.
[14] Lehrer RI, Lichtenstein AK, Ganz T. Defensins: antimicrobial and cytotoxic peptides of mammalian cells. Annu Rev Immunol 1993;11:105–28.
[15] Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol 2008;8(5):337–48.
[16] Aggarwal S, Gupta S, Agrawal A. Human dendritic cells activated via dectin-1 are efficient at priming Th17, cytotoxic CD8 T and B cell responses. PLoS One. 2010; 5(10):e13418; page 1–8.
[17] Kuvibidila S, Korlagunta K. Extracts from culinary medicinal mushrooms increase intracellular alpha-defensins 1-3 concentration in HL60 cells. International Journal of Medicinal Mushrooms 2010;12(1):33–41.
[18] Brown GD, Taylor PR, Reid DM, Willment JA, Williams DL, Martinez-Pomares L, et al. Dectin-1 is a major beta-glucan receptor on macrophages. J Exp Med 2002;196(3):407–12.
[19] Taylor PR, Brown GD, Reid DM, Willment JA, Martinez-Pomares L, Gordon S, et al. The beta-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. J Immunol 2002;169(7):3876–82.
[20] Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. J Nutr 1977;107:1340–8.
[21] Kim JJ, Shajib MS, Manocha MM, Khan WI. Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp 2012(60)pii: 3678.
[22] Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenter 1990;98(3):694–702.
[23] Vowinkel T, Mori M, Krieglstein CF, Russell J, Saijo F, Bharwani S, et al. Apolipoprotein A-IV inhibits experimental colitis. J Clin Invest 2004;114(2):260–9.
[24] Lee IA, Bae EA, Hyun YJ, Kim DH. Dextran sulfate sodium and 2,4,6-trinitrobenzene sulfonic acid induce lipid peroxidation by the proliferation of intestinal gram-negative bacteria in mice. J Inflam 2010;7:7.
[25] Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000;13(5):715–25.
[26] Higashi T, Hashimoto K, Takagi R, Mizuno Y, Okazaki Y, Tanaka Y, et al. Curdlan induces DC-mediated Th17 polarization via Jagged1 activation in human dendritic cells. Allergol Intern 2010;59:161–6.
[27] Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction on inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med 2003;197(9): 1107–17.
[28] Wang J, Ma J, Charboneau R, Barke R, Roy S. Morphine inhibits murine dendritic cell IL-23 production by modulating Toll-like receptor 2 and Nod2 signaling. J Biol Chem 2011;286(12): 10225–32.
[29] Chandra CL, Alexander H, Traore D, Lucas EA, Clarke SL, Smith BJ, et al. White button and shiitake mushrooms reduce incidence and severity of collagen-induced arthritis in dilute brown non-agouti mice. J Nutr 2010;141:131–6.
[30] Xu H, Ploplis VA, Castellino FJ. A coagulation factor VII deficiency protects against acute inflammatory responses in mice. J Pathol 2006;210(4):488–96.
[31] Robbins Pathological basis of disease. 6th edition Pp 260
[32] Chandra LC, Smith BJ, Clarke SL, Marlow D, D'Offay JM, Kuvibidila SR. Differential effects of shiitake- and white button mushroom-supplemented diets on hepatic steatosis in C57BL/6 mice. Food Chem Toxicol 2011;49(12):3074–80.
[33] Kuo SH, Lin LI, Chang CJ, Liu YR, Lin KS, Cheng AL. Increased risk of parvovirus B19 infection in young adult cancer patients receiving multiple courses of chemotherapy. J Clin Microbiol 2002;40(11):3900–12.
[34] Borchers AT, Krishnamurithy A, Keen CL, Meyers F, Grshiwin AE. The immunobiology of mushrooms. Exp Biol Med 2008;233:259–76.
[35] Lull C, Wichers HJ, Savelkoul HEJ. Antiinflammatory and immunomodulating properties of fungal metabolites. Mediat Inflamm 2005;2:63–80.
[36] Cunin P, Caillon A, Corvaisier M, Garo E, Scotet M, Blanchard S, et al. The tachykinins substance P and hemokinin-1 favor the generation of human memory Th17 cells by inducing IL-1 {beta}, IL-23, and TNF-like 1A expression by monocytes. J Immunol 2011;186(7):4175–82.
[37] Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat". Nat Immunol 2008;9(6):641–9.
[38] Marrazzo G, Bellner L, Halilovic A, Li Volti G, Drago F, Dunn MW, et al. The role of neutrophils in corneal wound healing in HO-2 null mice. PLoS One 2011;6(6):e21180.
[39] Kojima M, Nakamura N, Itoh H, Motoori T, Hoshi K, Enomoto Y, et al. Histological variety of localized lymphoid hyperplasia of the large intestine: histopathological, immunohistochemical and genotypic findings of 16 cases. J Clin Exp Hematop 2009;49(1):15–21.
[40] Jia Q, Ivanov I, Zlatev ZZ, Alaniz RC, Weeks BR, Callaway ES, et al. Dietary fish oil and curcumin combine to modulate colonic cytokinetics and gene expression in dextran sodium sulphate–treated mice. Br J Nutr 2011;106(4):519–29.