Brewer’s Yeast Supplementation Enhances Immune Response of Aged Mice

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A large amount of evidence indicates that aging is associated with immune system dysfunction. Brewer’s yeast, one of the most notable species of yeast in health and wellness, has been shown to stimulate the immune system and improve human life span. In the present study, brewer’s yeast (5, 25 or 125 μg/mouse) was orally supplemented to aged mice each other day for 4 weeks. Total number of leukocytes from peripheral blood (PBl), thymus (Thy), spleen (Sp), and peritoneal exudate cells (PEC) were counted. Carbon clearance, plaque-forming cell and rosette-forming cell assays were used to measure macrophage, B cell and T cell responses. In vitro proliferation of splenocytes and histological architecture of thymus from aged mice were tested. Yeast treated mice showed a significant increase in the total number of PBl leukocytes, total cellularity of both Thy and Sp, as well as a significant increase in total and differential numbers of PEC. A gradual increase in macrophages’ phagocytic activity of PEC and a significant increase in both plaque-forming cell and rosette-forming cell responses were observed. In vitro results showed that incubation of splenocytes with brewer’s yeast (0.5, 2.5 or 12.5 μg/well) in the presence of concanavalin-A mitogen elicited a gradual increase in the proliferation of splenocytes. Histological examination of thymuses from yeast-treated aged mice revealed that the cortex was preferentially enlarged and repopulated with immature thymocytes. These results indicate that brewer’s yeast supplementation to aged mice improves several baseline immune responses, and provides a valuable tool to identify nutritional strategies that could mitigate immunosenescence.

Aging is a complex process of living organisms. The age-related changes are well exemplified in the immune system, which contributes to the increased incidence of infectious and neoplastic diseases as well as to the prolonged periods of recovery after illness and greater morbidity observed in elderly subjects (O’Connor et al., 2014). Age-related functional changes have been well defined for both humoral and cell-mediated immune responses. Age-related T-cell immune deficiency is preceded by thymic involution and is influenced by several intrinsic as well as extrinsic factors (Holländer et al., 2010). Further, the role of monocytes and macrophages in T-cell activation changes with advancing age (Wang & Casolaro, 2014). Another important aspect of the age-associated changes in immune function is a decrease in both primary and secondary antibody responses (Simioni et al., 2007).

Yeast is the term generally applied to unicellular fungus. One of the most well-known species of yeast in health and wellness is known as Saccharomyces cerevisiae, which is also known by its more common name, brewer’s yeast. It is generally half composed of protein, as well as large amount of B vitamins, minerals and a diverse number of other healthy compounds. Typically, Brewer’s yeast is used as a protein supplement, energy booster or immune enhancer (Moyad, 2008).

Different studies have focused on the immunoenhancing properties of brewer’s yeast in adults, both in vivo and in vitro. A beta-glucan extracted from S. cerevisiae improved the humoral immunity and modulated the cellular immunity of weaned piglets (Li et al., 2005), inhibited metastasis and tumor growth in mice (Yoon et al., 2008), stimulated cytokine secretion from human neutrophil-like HL-60 cells (Saegusa et al., 2009), increased salivary IgA and decreased
cold/flu symptoms in human (McFarlin et al.,
2013), induced IFN-gamma production in vivo in mice (Javmen et al.,
2015), and enhanced both humoral and mucosal immunity and modulated uterine inflammatory signals in dairy cows (Yuan et al.,
2015). Brewer's yeast could serve as a vaccine adjuvant to enhance specific immune response (Berner et al.,
2008). Wade et al. (2010) also determined the potential value of S. cerevisiae as a host for cancer vaccine development. Recently, Shouval et al. (2015) suggested the efficacy and safety of recombinant yeast-derived hepatitis B vaccination for prevention of hepatitis B.

Considering the immunoenhancing activities of brewer's yeast supplementation in adult individuals, the relatively few clinical studies on the effect of brewer's yeast on the immune response of the elderly, and in order to confirm the usage of yeast-based technology in improving human life span, the present study is conducted to determine the in vivo effect of different doses of brewer's yeast supplementation on both cell-mediated and humoral immune responses of aged mice, as well as the in vitro effect of brewer's yeast on mitogen-induced proliferation from old mice splenocytes.

Materials and Methods

Animals
The experimental animals used in this study were outbreeding aged (18 months old) mice. Mice were obtained from El-Mansora Research Animal Center, Egypt. Animals were maintained in a quite room at 28°C with a light period of 12 hours alternating with a dark period of 12 hours. Mice received food and water ad libitum and were allowed a period of 10 days, prior to initiation of experiments, to acclimatize to the laboratory conditions.

Brewer's Yeast Treatment
Brewer's yeast tablets (MEPACO, Medi-Food, Egypt) were dissolved in a physiological saline, and doses of either 5, or 25 or 125 μg/mouse were administered orally to aged mice every other day for 4 weeks. Control aged mice were given each 0.2 ml of the physiological saline vehicle only.

Experimental Design
A total of 72 aged mice were used in this study. Mice were divided into 4 groups consisting of 18 mice each, one control group received 0.2 ml of physiological saline/mouse, and 3 groups treated with either 5, or 25 or 125 μg/mouse of brewer's yeast each other day for consecutive 4 weeks. Each group was further subdivided into 3 subgroups consisting of 6 mice each to perform the 3 experiments including total leukocytes count, carbon clearance assay, as well as plaque-forming and rosette-forming cells assays. All mice were sacrificed 24 hours after the last treatment.

Determination of Leukocytes Count In Lymphoid Organs
Thymuses (Thy) and spleens (Spl) of control and brewer's yeast-treated aged mice were excised, cleaned, defatted and weighed. Single cell suspensions of thymocytes and splenocytes were prepared by gentle squeezing of the respective organs between two slides in Hank's balanced salt solution (HBSS), followed by filtering through a nylon sieve. Red blood cells from these tissues, in addition to peripheral blood (PBl) were lysed by addition of Tris/NH4Cl buffer (0.017 M Tris-hydroxymethyl aminomethane and 0.16 M NH4Cl, pH 7.2). The respective cell suspensions were washed three times, resuspended in HBSS, and cells were counted with a haemocytometer and calculated per gram of tissues.

Harvesting of Peritoneal Exudate Cells
To obtain inflammatory peritoneal phagocytes, control and brewer's yeast-treated aged mice were intraperitoneally (i.p.) injected with 2.0 ml of starch suspension. Three days later, mice were sacrificed and the peritoneal exudate cells (PEC) were obtained by peritoneal lavage with 5.0 ml of HBSS. Cells were washed three times by centrifugation with 5.0 ml of HBSS. Cells were washed three times by centrifugation (160 r.p.m. for 10 min.) and resuspended in HBSS. Total and differential counts of PEC were determined using haemocytometer, by the uptake of 1 % W/V neutral red in saline (Hudson & Hay, 1989).

Carbon Clearance Assay
Phagocytic activity of PEC was measured by using Pelikan special biological ink (Pelikan-Werke, Hannover, Germany). The original suspension was diluted 1:1 with 0.9 % NaCl solution, and 0.2 ml of the diluted ink was i.p. injected into control and brewer's yeast-treated aged mice after stimulation with 2.0 ml of starch suspension, which was i.p. injected three days
earlier. Carbon challenged mice were sacrificed an hour after carbon injection. Five ml of 0.1 % EDTA-saline solution was i.p. injected, and peritoneal lavage was collected and centrifuged (700 r.p.m. for 5 min.). The resultant supernatant was decanted into another tube, and the precipitated cells were resuspended in 1 ml of equal volumes of gelatin (2 % gelatin in saline) and ethanol potassium saline (5 % KOH in 70 % ethanol), and incubated overnight at 37 °C. Optical densities of both supernatant and digested cells were measured using a spectrophotometer (Spectronic 20, Bausch and Lomb Inc., Rochester, NY, USA).

Detection of Plaque-Forming Cells (PFC)

The procedure was performed as described by Brousseau et al. (1999). Primary humoral immune responses against sheep red blood cells (SRBC) were measured in control and brewer's yeast-treated aged mice after one i.p. injection of 1x10^8 SRBC in 0.2 ml saline. Five days later, mice were sacrificed and spleens were excised and cleaned. Single cell suspensions were prepared, washed twice by centrifugation at 200 g and resuspended in HBSS to a concentration of 2x10^6/ml. The liquid assay mixture was prepared by adding 50 µl of 25 % SRBC and 50 µl of guinea pig complement to 100 µl of spleen cell suspension. The assay mixture was plated to a slide chamber and incubated for 30 to 45 min. at 37°C. The plaques were scored microscopically and calculated per million mononuclear cells.

Detection of E Rosette-Forming Lymphocytes

The procedure was performed as described by Hsu et al. (1975). Five days before they were sacrificed, control and brewer's yeast-treated aged mice received an i.p. injection of 1x10^8 SRBC in 0.2 ml saline. Spleens were excised; cleaned and single cell suspensions were prepared, washed twice by centrifugation at 200g and resuspended in HBSS to a concentration of 2x10^6/ml. 0.2 ml of spleen cell suspension was mixed with an equal volume of 0.5 % SRBC in a glass tube and incubated for 2-4 hours at 37 °C. The tubes containing the mixture were gently shaken to resuspend the cells in the pellet. The rosettes were counted in a haemocytometer and calculated per million mononuclear cells. The cells surrounded by three or more SRBC were counted as E-rosette-forming T-cells.

Culture Procedure

Aged mice were sacrificed by cervical dislocation and spleens were aseptically removed and pooled. Spleens were dispersed gently by using two sharp forceps in serum free RPMI-1640 medium. The splenocytes were then seeded into 96-well culture plates (Falcon, Oxnard, CA) at a density of 1.5 x 10^3 splenocytes/well in RPMI-1640 medium supplemented with 5 % fetal calf serum (Gibco, Grand Island, N.Y.), 50 µM β-mercaptoethanol (Sigma Chemical Co.) and antibiotics. The cells were treated with 0.04 µg/well concanavalin A (Con A) in the presence of 0.5, 2.5 or 12.5 µg/well brewer's yeast and subsequently incubated in a humidified 5 % CO₂ environment. Three days later, 150 µl of the medium was removed from each well. The extent of spleen cell proliferation was determined using the tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, Sigma Chemical Co.), which adheres to active mitochondria to form a dark blue formazan product (Mosmann, 1983). MTT (5 µl of 5 mg/ml) was added to each well and incubated at 37 °C for 4 hours. The dark blue crystals were dissolved by the addition of 150 µl of 0.04 M HCl/isopropanol. After an overnight incubation in the dark, the plates were inserted into a Dynatech MR580 microelisa spectrophotometer and optical densities were obtained using a test wavelength of 570 nm and a reference wavelength of 630 nm.

Histological Study

Control and brewer's yeast-treated aged mice were sacrificed 24 hours after the last treatment. Thymuses were excised and fixed in 10 % neutral buffered formalin. The specimens were then dehydrated in ascending grades of ethyl alcohol, cleared in terpinol, washed in benzene, embedded in paraffin wax, sectioned at 5 µ, and stained with haematoxylin and eosin (Delafield, 1984).

Statistical Analysis

All in vivo data are expressed as the mean ± SD of groups consisting of 6 mice. The in vitro data are expressed as the mean ± SD of groups consisting of four wells. Each experiment was performed independently at least three times. All data were analysed for significance using Student’s t-test. Values of P < 0.05 and P < 0.01 were considered statistically significant.

Results

Effect of Aging and Brewer's Yeast Treatment on Lymphoid Cell Count

Table 1 shows that treatment of aged mice with brewer's yeast (5, 25 or 125 µg/mouse every other day for 4 weeks) elicited a statistically significant increase in the number
of PBI and Thy leukocytes at $P < 0.05$ with the dose 5 μg/mouse, and at $P < 0.01$ with the doses 25 and 125 μg/mouse, when compared with that of control aged mice. On the other hand, the number of Sp leukocytes was slightly increased with the dose 5 μg/mouse, and significantly increased at $P < 0.05$ and $P < 0.01$ with the doses 25 and 125 μg/mouse, respectively, when compared with that of control aged mice.

Table 1. Total leukocytes from peripheral blood, thymus and spleen in control and brewer's yeast-treated aged mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total leukocytes count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBl/mm$^3 \times 10^6$</td>
</tr>
<tr>
<td>Control aged mice</td>
<td>4.28±0.73</td>
</tr>
<tr>
<td>Yeast-treated aged mice (5 μg)</td>
<td>5.98±1.14*</td>
</tr>
<tr>
<td>Yeast-treated aged mice (25 μg)</td>
<td>7.85±1.25**</td>
</tr>
<tr>
<td>Yeast-treated aged mice (125 μg)</td>
<td>7.53±1.61**</td>
</tr>
</tbody>
</table>

The total number of leukocytes from peripheral blood (PBI), thymus (Thy) and spleen (Spl) in control and brewer's yeast-treated (5, 25 or 125 μg/mouse) aged mice. The data are expressed as the mean ± SD of groups consisting of 6 mice. (*: at $P < 0.05$ and **: at $P < 0.01$ in comparison with the control aged group)

Effect of Aging and Brewer's Yeast Treatment on Total and Differential PEC Counts

As shown in Table 2, treatment of aged mice with brewer's yeast (5, 25 or 125 μg/mouse every other day for 4 weeks) elicited a statistically significant increase in the total number of PEC as well as the absolute number of both peritoneal macrophages and lymphocytes at $P < 0.05$ with the dose 5 μg/mouse, and at $P < 0.01$ with the doses 25 and 125 μg/mouse, when compared with that of control aged mice.

Table 2. Total and differential peritoneal exudate cells count in control and brewer's yeast-treated aged mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total PEC count $x10^6$</th>
<th>Macrophages $x10^6$</th>
<th>Lymphocytes $x10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute No.</td>
<td>%</td>
<td>Absolute No.</td>
</tr>
<tr>
<td>Control aged mice</td>
<td>3.66±0.68</td>
<td>2.34±0.33</td>
<td>63.9</td>
</tr>
<tr>
<td>Yeast-treated aged mice (5 μg)</td>
<td>5.13±1.09*</td>
<td>3.07±0.56*</td>
<td>59.8</td>
</tr>
<tr>
<td>Yeast-treated aged mice (25 μg)</td>
<td>6.81±1.32**</td>
<td>4.33±0.97**</td>
<td>63.6</td>
</tr>
<tr>
<td>Yeast-treated aged mice (125 μg)</td>
<td>7.88±1.39**</td>
<td>5.08±1.10**</td>
<td>64.5</td>
</tr>
</tbody>
</table>

The total peritoneal exudate cell (PEC) count, absolute number and relative proportion (%) of both macrophages and lymphocytes in control and brewer's yeast-treated (5, 25 or 125 μg/mouse) aged mice. The data are expressed as the mean ± SD of groups consisting of 6 mice. (*: at $P < 0.05$ and **: at $P < 0.01$ in comparison with the aged control group)

Effect of Aging and Brewer's Yeast Treatment on Phagocytic Function of PEC

To study the phagocytic function of PEC, carbon uptake by PEC as well as carbon particles remained in peritoneal fluid were measured in control and brewer's yeast-treated aged mice after an hour of injection. Table 3 shows that treatment of aged mice with brewer's yeast (5, 25 or 125 μg/mouse every other day for 4 weeks) elicited a gradual increase in the scavenger activity of PEC. This increase was insignificant with the dose 5 μg/mouse, statistically significant, at $P < 0.05$, with the dose 25 μg/mouse, and
Effect of Aging and Brewer’s Yeast Treatment on B cell Function

The number of plaque-forming cells (PFCs)/million splenocytes was determined 5 days after i.p. immunization of control and brewer’s yeast-treated aged mice with 1x10⁸ SRBC. As shown in Table 4, treatment of aged mice with brewer’s yeast (5, 25 or 125 µg/mouse every other day for 4 weeks) elicited a marked increase in the number of PFCs. This increase was insignificant with the dose 5 µg/mouse, and statistically significant, at P<0.05 and at P<0.01, with the doses 25 and 125 µg/mouse, respectively, when compared with that of control aged mice.

Effect of Aging and Brewer’s Yeast Treatment on T cell Number

The number of rosette-forming cells (RFCs)/million splenocytes was determined 5 days after i.p. immunization of control and brewer’s yeast-treated aged mice with 1x10⁸ SRBC. As shown in Table 4, treatment of aged mice with brewer’s yeast (5, 25 or 125 µg/mouse every other day for 4 weeks) elicited a marked increase in the number of RFCs. This increase was statistically significant at P<0.05 with the dose 5 µg/mouse, and at P<0.01, with the doses 25 and 125 µg/mouse, respectively, when compared with that of control aged mice.

Table 3. Phagocytic activity of peritoneal exudate cells in control and brewer’s yeast-treated aged mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carbon uptake by PEC (O.D. after an hour)</th>
<th>Carbon particles remained in fluid (O.D. after an hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control aged mice</td>
<td>0.89 ± 0.09</td>
<td>1.03 ± 0.12</td>
</tr>
<tr>
<td>Yeast-treated aged mice (5 µg)</td>
<td>0.99 ± 0.10</td>
<td>0.91 ± 0.11</td>
</tr>
<tr>
<td>Yeast-treated aged mice (25 µg)</td>
<td>1.10 ± 0.14*</td>
<td>0.86 ± 0.09*</td>
</tr>
<tr>
<td>Yeast-treated aged mice (125 µg)</td>
<td>1.27 ± 0.14**</td>
<td>0.79 ± 0.08**</td>
</tr>
</tbody>
</table>

Table 4. B and T lymphocytes responses in control and brewer’s yeast-treated aged mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of PFCs (10³/10⁶ nucleated spleen cells)</th>
<th>No. of RFCs (10³/10⁶ nucleated spleen cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control aged mice</td>
<td>0.81 ± 0.11</td>
<td>1.77 ± 0.29</td>
</tr>
<tr>
<td>Yeast-treated aged mice (5 µg)</td>
<td>0.96 ± 0.12</td>
<td>2.49 ± 0.44*</td>
</tr>
<tr>
<td>Yeast-treated aged mice (25 µg)</td>
<td>1.02 ± 0.15*</td>
<td>3.07 ± 0.53**</td>
</tr>
<tr>
<td>Yeast-treated aged mice (125 µg)</td>
<td>1.73 ± 0.34**</td>
<td>3.24 ± 0.66**</td>
</tr>
</tbody>
</table>
In vitro Effect of Brewer's Yeast Treatment on T Cell Mitogenesis of Aged Mice

In order to examine the in vitro effect of brewer's yeast on concanavalin A (Con A)-stimulated splenocytes from aged mice, cultured splenocytes (1.5x10^5 splenocytes/well) were exposed to culture medium (Control) and Con A (0.04 µg/well) in the presence of brewer's yeast (0.5, 2.5 and 12.5 µg/well) and incubated for 72 hours. As shown in Table 5, in the absence of Con A, brewer's yeast by itself elicited a gradual increase in the proliferation of splenocytes. This increase was statistically significant at \( P < 0.05 \) with the higher dose (12.5 µg/well), when compared with that of control aged mice. In the presence of Con A, brewer's yeast elicited a gradual increase in the proliferation of splenocytes. However, this increase was statistically significant at \( P < 0.05 \) with the dose 2.5 µg/well and at \( P < 0.01 \) with the dose 12.5 µg/well, when compared with that of control aged mice.

Table 5. In vitro effect of brewer's yeast on concanavalin A (Con-A)-stimulated splenocytes from aged-mice.

<table>
<thead>
<tr>
<th>Brewer's yeast (µg/well)</th>
<th>Optical density (O.D.) at 570 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Cultured medium)</td>
</tr>
<tr>
<td>0</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>0.5</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>2.5</td>
<td>0.31 ± 0.05</td>
</tr>
<tr>
<td>12.5</td>
<td>0.39 ± 0.08*</td>
</tr>
</tbody>
</table>

Cultured murine splenocytes (1.5x10^5 cells/well) were exposed to culture medium (Control) or Con A mitogen (0.04 µg/well) in the absence or presence of brewer's yeast (0.5, 2.5 and 12.5 µg/well) for 72 h. The data are expressed as the mean ± SD of groups consisting of four wells. (*: at \( P < 0.05 \) and **: at \( P < 0.01 \) in comparison with splenocytes from control aged group).

Histological Examination of the Thymus Cortex

Figure 1 showed the histological architecture of the thymus cortex in control and yeast-treated aged mice. Figure 1 a, the thymus cortex of control aged mice revealed light aggregations of immature thymocytes. Figure 1 b, c and d, the thymus cortex of brewer's yeast-treated aged mice (5, 25 or 125 µg/mouse) exhibited a gradual increase in the number of immature thymocytes, which became normal in appearance with the higher dose of brewer's yeast.
Figure 1. Histological architecture of the thymus cortex in control aged and yeast-treated aged mice. (a) The thymus cortex of control aged mice showed the decline in thymocyte (Thy) production; (b, c and d) The thymus cortex of brewer's-treated aged mice (5, 25 and 125 μg/mouse) showed the gradual increase in the number of thymocytes (H&E, X 400).
Discussion
The present study showed that aging was accompanied by a significant decrease in the number of leukocytes from PBl, Thy, and Spl, as well as total and differential counts of PEC in mice. Treatment of aged mice with brewer's yeast caused a progressive increase in the total number of leukocytes from all these lymphoid tissues. These results are consistent with the results of previous studies. Aging is associated with a significant decrease in peripheral leukocytes (Izumi-Hisha et al., 1990), a marked decrease in the total mononuclear cells in the thymus (Marusić et al., 1998), a substantial decline in the number of splenocytes (Kawanishi & Kiely, 1989), and a significant decrease in the total and differential count of PEC (El-Shaikh et al., 2006). Brewer's yeast treatment showed beneficial effects on leukocytes counts in different animal models. Brewer's yeast supplementation increased the total number of white blood cells in carp (Selvaraj et al. 2005), in breeding hens (Matur et al., 2011), and in fingerlings (Andrews et al., 2011). A single dose of *S. cerevisiae* also increased the number of PECs in gilthead seabream fish (Andrews et al., 2011).

The present work shows that phagocytosis by peritoneal macrophages of aged mice was significantly decreased as compared with those of both young and adult mice. However, treatment of aged mice with brewer's yeast elicited a progressive increase in the scavenger activity of peritoneal macrophages. The present result agrees with the result of Sharma et al. (2014) who reported a remarkable decrease in the phagocytic activity of neutrophils by aging. The present result also confirms the result of Gue et al. (2003) who reported that yeast beta-glucan supplementation increased the macrophage phagocytic activity in broiler chicks. However, Seong & Kim (2010) demonstrated the presence of *S. cerevisiae* beta-glucan receptor on murine macrophages, suggesting a mechanism of immune-potentiation by beta-glucan.

The present study showed that the number of PFCs of aged mice was significantly less than that of both young and adult mice. Treatment of aged mice with brewer's yeast caused a significant increase in PFC response. This result confirms the result of Attia et al. (2002) who reported a decrease in the anti-SRBCs antibody response in aged mice, and the result of Gibson et al. (2009) reported that aging is associated with a decline in diversity and antigen-specificity of circulating B cells. The present result also confirms the result of Gue et al. (2003) reported that yeast beta-glucan supplementation increased the anti-SRBCs antibody response in broiler chicks.

The present study showed that the number of T cells of aged mice was significantly decreased when compared with that of young and aged mice. Treatment of aged mice with brewer's yeast significantly increased the number of T cells. There is a considerable body of literature demonstrating that cell-mediated immune responses are impaired in elderly individuals. The rosette-forming T cell response (El-Shaikh et al., 2006), the circulating numbers of naïve CD8+ cytotoxic T cells are reduced and diversity of T cell receptors (Goronzy et al., 2007) and stimulation index and CD28 expression in T lymphocytes (Sharma et al., 2014) are decreased with aging. However, brewer's yeast treatment showed beneficial effects on T lymphocytes. Brewer's yeast supplementation increased the percentage of CD4++ , CD8++, and CD4+/CD8+ double positive lymphocytes in the intestinal intraepithelial leukocytes of broiler chicks (Gue et al., 2003), increased T lymphocyte ratio in the spleen of mice (Jung et al., 2010) and provided a rapid and transient effect on the trafficking and
activation status of specific lymphocyte subsets in vitro (Jensen et al., 2011).

The ability of brewer's yeast to elicit a lymphoproliferative response of aged mice splenocytes was examined in the present study. Brewer's yeast significantly stimulated the mitogenesis of splenocytes from aged mice in the presence of Con A mitogen. The present result confirms the results of Douziech et al. (2002) who previously reported that the proliferative response to stimulation with phytohaemagglutin mitogen is greater in young than old human, and the result of Darroch et al. (1994) who observed a dose-dependent lymphoproliferative response of human lymphocytes to a purified glycoprotein from S. cerevisiae and the results of Malaczewksa & Milewski (2010) who found a higher proliferative response of blood lymphocytes after stimulation with LPS and Con A in brewer's yeast-treated lambs. Zhang et al. (2012) also reported that S. cerevisiae cell wall extract mitigated the cyclosporine A-induced PBI lymphocyte blastogenic response. The work of Ibrahimo et al. (2006) supports a model where the yeast binding proteins are acting on specific mRNAs to facilitate a defined proliferative response.

In the present study, histological examination of the thymus during progression of aging revealed a preferential involution of the cortex with few numbers of thymocytes. Treatment of aged mice with brewer's yeast exhibited enlarged cortex which repopulated with large number of immature thymocytes. It is well documented that chronic involution of the thymus is thought to be one of the major contributing factors to the loss of immune function with aging. Thymic involution leads to impaired T cell differentiation and maturation with aging resulting in a significant decline in the output of new T cells (Miller, 1996; Webster, 2000). However, Holländer et al., (2010) attributed the decline of immunocompetence with aging to the involution and atrophy of the thymic gland, which results in alterations in lymphoid cell number and function. Previous study by Gue et al. (2003) demonstrated that the thymus of yeast beta-glucan supplemented chicken was larger as compared with the chicks supplemented with basal diet. Morales-Lopez et al. (2009) also reported that broilers fed dietary addition of yeast cell wall had greater thymus weights as compared with control. Seong and Kim (2010) demonstrated the presence of S. cerevisiae beta-glucan receptor on murine mucosal organs such as the thymus, suggesting a mechanism of immunopotentiation by beta-glucan.

In conclusion, the present results indicate that brewer's yeast supplementation has beneficial effects in strengthening the living body's immune system of aged mice. Brewer's yeast supplementation to aged mice increased the total number of leukocytes from PBl, Thy, and Spl, as well as the total and differential counts of PEC. Brewer's yeast also enhanced macrophage function, increased the number and activity of T and B cells, and augmented responses of splenocytes to Con A mitogen. Therefore, brewer's yeast nutrition in old age may provide a valuable tool to identify nutritional strategies that could mitigate immunosenescence.

References


