ORIGINAL

Soy isoflavone, Soyaflavone HG, improves low T cell proliferation response in aged BALB/c mice

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Abstract : Aging is a phenomenon in which cells, tissues and organs undergo systemic pathological changes. We examined the effects of soy isoflavone on immune function in aged mice. Although aged mice showed lower T cell response against anti-CD3 monoclonal antibody stimulation than did young mice, aged mice have treated with soy isoflavone reversed the low responses. The percentage of $CD4^+$ cells was higher in aged mouse splenocytes treated with soy isoflavone than in untreated mouse splenocytes. Unexpectantly, aged mice treated with soy isoflavone showed higher programmed cell death-1 expression on T cells than did aged control mice. These results showed that soy isoflavone improve low T cell proliferation response in aged mice. J. Med. Invest. 73 : 212-216, February, 2026

Keywords **:** *soy isoflavone, Soyaflavone HG, aging, T cell function, PD-1*

INTRODUCTION

Aging is a phenomenon in which cells, tissues and organs undergo systemic pathological changes that promote the occurrence of aging-related diseases and the end of life (1). The increasing number of the aged population has become serious issue in many developed countries globally. Estimates in Japan show that 36 million people are over 65 years, accounting for 29.1% of the population (2). Although Japan has one of longest average lifespan in the world, certain problems including medical cost and health status, have been emerging (2). Thus, the promotion of research aimed at preventing aging has become imperative.

Many Asian countries have traditionally consumed soy foods (3). Soy contains some functional components and has shown positive effects against certain diseases including osteoporosis (4, 5), dyslipidemia (6, 7) and malignant cancer (8, 9). Soy isoflavones are one of the components of soy that contribute to its health benefits. Human intervention studies have been conducted and shown that soy components have positive effects for human health (10-12).

Genistein and daidzein are most studied soy isoflavones in the field of immunology. Our previous studies have shown that genistein exerts immunomodulatory actions in antigen-immunized mice (13), mouse models of atopic dermatitis (14), and mouse models of dextran sulfate sodium salt-induced colitis (15).

The age-associated $CD8^+$ T cells exhibit a phenotype of T cell exhaustion characterized by high expression levels of TOX, programmed cell death-1 (PD-1), lymphocyte activation gene 3 protein (LAG3) and T cell receptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains, which generally suggests a loss of classical effector functions (16). Although the concept of T cell exhaustion is less well established for $CD4^+$ T cells than for $CD8^+$ T cells, an age-associated increase in the abundance of $CD4^+$ T cells with high levels of inhibitory receptors, PD-1 and LAG3, has been shown (16).

The current study examined the effects of soy isoflavones on immune cell function in aged mice and found that they were effective for aging-related immune dysfunction.

MATERIALS AND METHODS

Mice

Female BALB/c mice (6 and 46 weeks old) were maintained in under a specific pathogen-free condition with a 12-hr light : dark cycle at 25±2°C and 55±10% relative humidity. The mice were given free access to water and food throughout the experiment. The mice were maintained on a control diet (No. D10012G ; Research Diets Inc., NJ, USA). All experiments were performed in accordance with the ethical guidelines for animal experimentation by the Institute of Biomedical Sciences, Tokushima University, Japan and were approved by the review board of the animal ethics committee.

Treatment with soy isoflavones

Soy isoflavone Soyaflavone HG was provided from Fuji Oil Ltd. (Osaka, Japan). The contents of each isoflavone in the Soyaflavone HG is detailed in Table 1. Soy isoflavone was dissolved in water at 0.5% (w/w) and were administered to the mice by drinking water.

T cell proliferation response

Splenocytes (5 x 10^5/well) were stimulated with anti-mouse CD3 monoclonal (m) antibody (Ab) (plate coated with 50 µl solution over night at a concentration of 1 µg/ml in phosphate-buffered saline) in a 96-well flat-bottom plate at 37°C under 5% CO_2 for 48 hr. For the last 8 hr of culture, 7.2 kBq of [^{3}H] thymidine-deoxyribose (TdR) was added to the wells, and the amount of [^{3}H]TdR incorporated was measured by a scintillation counter (Aloka, Tokyo, Japan).

IL-2 production

Splenocytes (2.5 x 10^6/well) were stimulated with anti-mouse CD3 mAb in a 48-well flat-bottom plate at 37°C under 5% CO_2 for 48 hr. Contents of IL-2 in the supernatants were quantified using mouse IL-2 ELISA kit (eBioscience, CA, USA) according to the instructions of the manufacturer.

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Table 1. Contents of soy isoflavones

Soy isoflavone	aglycone equivalent (%)
Total	26.02
Daidzin	4.10
Glycitin	3.46
Genistin	0.44
Malonyl Daidzin	11.39
Malonyl Glycitin	4.75
Malonyl Genistin	1.43
Acetyl Daidzin	0.22
Acetyl Glycitin	0.12
Acetyl Genistin	0.02
Daidzein	0.07
Glycitein	0.01
Genistein	0.01

Flow cytometry analysis

Splenocytes were stained with fluorescein isothiocyanate-conjugated anti-CD8 mAb, phycoerythrin-conjugated anti-CD4 mAb, peridinin-chlorophyll-protein-conjugated anti-PD-1 mAb and allophycocyanin-conjugated anti-B220 mAb. All of the Abs were purchased from eBioscience. Flow cytometry analysis was performed on Guava easyCyte using Guava Incyte software (Merck Millipore, Darmstadt, Germany).

Blood biochemical markers

Serum levels of total cholesterol (T-CHO), triglycerides (TG) and glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) were analyzed using enzymatic kits (Cholesterol E test Wako, Triglyceride E test Wako and GOT/GPT test Wako ; Wako Pure Chemical Industries, Osaka, Japan).

Statistics

Data are shown as means ± standard deviation. The results for aged control and soy isoflavone groups were compared using the t-test. A p-value of < 0.05 indicates statistical significance.

RESULTS

Blood biochemical markers

After soy isoflavone administration for 4 months, blood biochemical markers were determined. T-CHO concentration in aged mice treated with soy isoflavone was lower than that in aged control mice. No difference in TG, GOT and GPT values were observed between the control and soy isoflavone groups (Table 2).

Table 2. Blood biochemical markers.

	Young	Aged control	Aged isoflavone
T-CHO (mg/dl)	118 ± 11.3[a]	125.7 ± 20.9	105.5 ± 14.2[b]
TG (mg/dl)	83.0 ± 6.3	85.6 ± 17.1	81.5 ± 10.0
GOT (IU/L)	69.1 ± 6.7	71.6 ± 13.1	81.0 ± 22.9
GPT (IU/L)	7.9 ± 1.4	5.5 ± 2.9	5.7 ± 2.1

[a] Mean ± SD
[b] $p < 0.05$ vs aged control

Soy isoflavone improves low T cell response in aged mice

We determined T cell proliferation responses by stimulating with anti-CD3 mAb. The T cell response to anti-CD3 mAb was weaker in aged mice than in young mice. However, soy isoflavone treatment in aged mice reversed the reduced T cell response (Figure 1A). We also determined IL-2 production as an activation marker. Although IL-2 production in aged mice tended to be higher than that in young mice, no significant difference was observed. No significant difference in the level of IL-2 production was observed between aged control and aged soy isoflavone groups (Figure 1B).

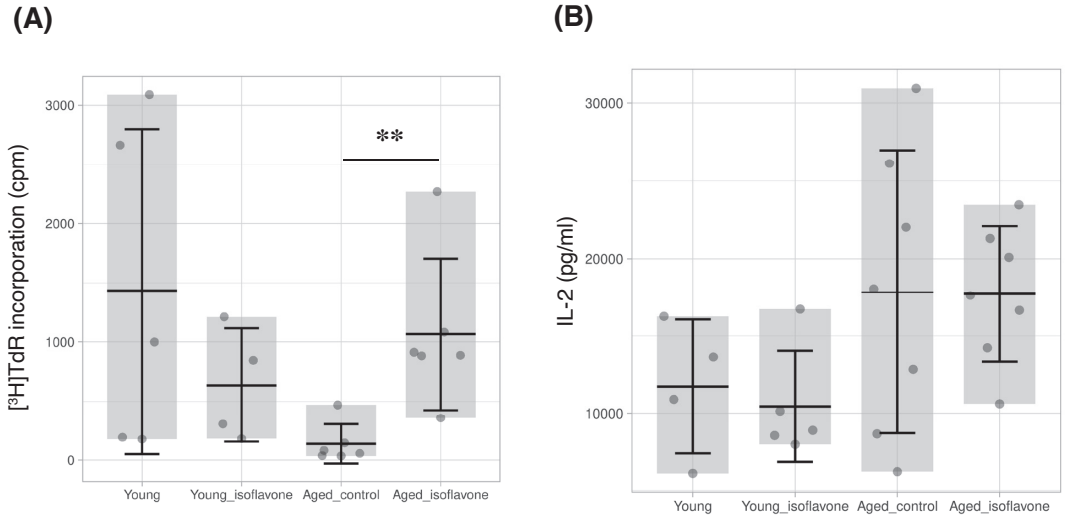

Figure 1. Soy isoflavone treatment enhances T cell response. Aged BALB/c mice were treated with or without soy isoflavone for four months. Splenocytes from these mice were stimulated with anti-CD3 mAb. Determinations of T cell response (A) and IL-2 production (B) were done as described in the Materials and Methods section. The young group, young isoflavone group, aged control group and aged isoflavone group consisted of five, four, six and seven mice, respectively. ** $p < 0.01$.

Soy isoflavone treatment increases the proportion of CD4$^+$ cells

To determine immune cell subsets in the spleen, flow cytometry analysis was conducted. The percentage of CD4$^+$ cells was higher in aged mice treated with soy isoflavone than in control aged mice. Although we determined the percentage of CD8$^+$ and B220$^+$ cells in aged mice treated with or without soy isoflavone, no difference was observed among these groups (Table 3).

Table 3. Percentages of CD4$^+$ and CD8$^+$ cells in splenocytes.

	Young	Aged control	Aged isoflavone
CD4$^+$ T cells (%)	31.6 ± 5.8[a]	28.0 ± 5.1	33.6 ± 1.5[b]
CD8$^+$ T cells (%)	12.7 ± 2.0	11.0 ± 1.6	10.3 ± 1.8
B220$^+$ cells (%)	41.9 ± 4.6	44.1 ± 5.3	45.5 ± 3.5

[a] Mean ± SD
[b] $p < 0.05$ vs aged control

Expression of PD-1 molecule in mice treated with soy isoflavone

Flow cytometry was performed to determine the expression of the PD-1 molecules, one of the markers of senescence in T cells. Aged mouse splenocytes tended to have an increased percentage of PD-1$^+$ cells compared to young mouse splenocytes. Unexpectedly, aged mice treated with soy isoflavone had a significantly higher percentage of PD-1$^+$ cells than did aged control mice (Table 4).

Table 4. Percentages of PD-1$^+$ cells in splenic CD4$^+$ and CD8$^+$ cells.

	Young	aged control	Aged isoflavone
CD4$^+$ T cells (%)	31.6 ± 18.2[a]	38.7 ± 12.9	55.5 ± 8.3[b]
CD8$^+$ T cells (%)	34.0 ± 20.3	39.7 ± 16.0	59.3 ± 9.8[b]

[a] Mean ± SD
[b] $p < 0.05$ vs aged control

DISCUSSION

Studies have shown that soy isoflavones exert various biological functions. The current study, we found that soy isoflavones were effective for reversing senescent immune cell function in aged mice (Fig. 1A). Evidence suggests that that T cell phenotype and function change with ageing (16). Regulatory T cells (Tregs) play a crucial role in modulating the immune response, and changes in their function with age directly affect susceptibility to autoimmune diseases, as well as the outcomes of infections and cancer. Studies have shown that Tregs accumulate in aged individuals (17, 18) and suppress T cell response against anti-CD3 mAb stimulation. One of the reasons for the recovery of reduced response in mice treated with soy isoflavone is the reduction of the number of Tregs. Unfortunately, we cannot address this point considering that the current study did not determine the proportion of CD4$^+$CD25$^+$Foxp3$^+$ cells. The level of proliferation was much higher in mice treated with soy isoflavone than in aged control mice (Fig. 1A). Changes in the population of Tregs might explained the recovery of proliferation response given the increase in the percentage of CD4$^+$ cells in aged mice after soy isoflavone treatment (Table 3). Hence, it should determine which CD4$^+$ cell subsets became altered by treatment with soy isoflavone.

After examining the production of IL-2 as an activation marker in the supernatants following anti-CD3 mAb stimulation, we found no significant difference in their levels between aged control and isoflavone groups (Fig. 1B). Given that T cell receptor-mediated T cell activation induces both cell proliferation and cytokine production, it remains undermined why soy isoflavones regulate only proliferation pathway. Effects of soy components on immune cell function have been examined in the senescence-accelerated-prone 8 (SAMP8) mice. SAMP8 mice has been shown to be markedly lower immune cell activities than their normal counterparts, senescence-accelerated-resistance 1 mice (19). Supplementation of nanonized black soybeans in SAMP8 mice enhanced lymphocyte proliferation response and cytokine productions (20). Therefore, it is possible that some soy components enhance lymphocyte proliferation in aged mice. Activated T cells produce IL-2 and use own IL-2 for their proliferation response. Therefore, it is possible that the contents of IL-2 are similar between control and isoflavone group because T cells from aged mice treated with soy isoflavone use more IL-2 for their proliferation than did T cells from aged control mice.

The soy isoflavones used in the current study were crudely isolated components. Thus, identifying which type of isoflavone was responsible for the enhancement of T cell response was difficult. Studies have shown that soy isoflavones suppress inflammatory and immune response. In particular, soy isoflavone genistein suppresses inflammatory cytokine productions and nitric oxide productions in macrophages and macrophage cell lines following lipopolysaccharide stimulation (21, 22). Genistein has been shown to inhibit antigen-specific humoral and cellular immune responses in ovalbumin-immunized mice (13). Studies have also demonstrated the suppressive effect of genistein in mouse model of immune diseases, including autoimmune encephalomyelitis (23) and atopic dermatitis (14). Based on our experiment, soy isoflavones cannot enhance T cell response in young mice (Fig. 1A). At the present study, it is difficult for discuss the mechanism why soy isoflavone enhance T cell response in aged mice. Therefore, the omics approach for mRNA and protein levels might be necessary to address this issue in soy isoflavone-treated mice.

T-CHO concentration in aged mice treated with soy isoflavone was lower than that in aged control mice (Table 2). It has been shown that soy isoflavone regulates lipid metabolism in mice and human (24-26). Although change of TG concentration was not observed in aged soy isoflavone group, the level of T-CHO might be sensitively response to the action of soy isoflavone.

To date, several markers of senescence have already been identified. Age induces the expression of p16, p21 and PD-L1 molecules at the cellular levels and organ levels (27). In T cells, it has been shown that exhausted-like PD-1$^+$ cells are accumulated in the spleen, peritoneum, lung and liver (16). Hence, the increased expression of PD-1 in the T cell of aged mice treated with soy isoflavone is unexpected given the enhanced T cell response observed herein (Table 4 and Fig. 1A). A significant reduction in T cell response was observed with age (Fig. 1A). No difference in the expression of p16 and p21 molecules in the lungs, liver and kidneys was observed between aged control and isoflavone-treated aged mice (Supplemental Fig. 1). These findings likely suggest that the senescence phenotype emerged relatively earlier during immune function with age.

Though little research has been conducted on the effects of soy isoflavones on aging and immunity, their impact on skin aging has been investigated (28, 29). Skin tissue is known to be fast turnover tissue. Indeed, available evidence suggests that soy isoflavones suppress ultraviolet radiation-induced epithelial thickening and DNA damage of the skin. Moreover, *in vitro* studies have found that genistein suppresses the production of IL-1, MIF, PLANH1 and H_2O_2 in ultraviolet radiation-exposed HaCaT cells (30). In the infection, T cells rapidly replicate in response to pathogens. It is possible that soy isoflavone

preferentially affect to the function of high-turnover cells.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Supplemental Table 1. Expressions of p16 and p21 mRNA in aged mice treated with or without soy isoflavone.

		Relative expression		
		Lung	Liver	Kidney
p16	Aged control	89.4 ± 75.6[a]	23.7 ± 17.3	4.8 ± 2.9
	Aged isoflavone	225.3 ± 129.1	25.9 ± 19.1	8.2 ± 3.4
p21	Aged control	4.5 ± 7.2	ND[b]	9.0 ± 3.3
	Aged isoflavone	5.4 ± 5.9	ND	12.4 ± 8.9

[a] Mean ± SD
[b] Not determined