REVIEW

Soy isoflavones and immunity

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Abstract : The amount of soy products consumed in Japan is much greater than that in Western countries. Recent evidence indicates that soy isoflavones play a beneficial role in obesity, cancer, osteoporosis, and cardiovascular disease. The soybean isoflavone genistein is present at high levels in soy products. Genistein is structurally similar to 17β-estradiol (E2), and genistein has been suggested to be act as E2 or an antagonist against E2. Genistein suppresses antigen-specific immune response *in vivo* and lymphocyte proliferation response *in vitro*. However, genistein enhances the cytotoxic response mediated by NK and cytotoxic T cells and the cytokine production from T cells. Thus, the effect of genistein on immunity is immune cell-dependent. Due to its unique effect on immune function, genistein has been used for the treatment of the diseases in animal models and it has been found that genistein inhibits allergic inflammatory responses. In this review, we summarize current studies related to the effect of isoflavone genistein on the immune system. J. Med. Invest. 55 : 167-173, August, 2008

Keywords **:** *soy isoflavone, genistein, immunity, T cell*

INTRODUCTION

The intake of diets low in fat and high in complex carbohydrates from grains, fruits, and vegetables is associated with a lower risk of chronic diseases (1). Although this has been suggested to be due to the adverse effect of fat and the potential health benefits of dietary fiber, other constituents associated with high-fiber foods may also be responsible in part for the health benefit of such diets. In recent years, phytoestrogens have been attracting increasing attention among the public and in the medical community because of evidence from a large body of literature suggesting that consumption of plant-based foods rich in these phytochemicals may benefit human health (1-8). Substantial data from epidemiologic surveys and nutritional intervention studies in humans and animals suggest that dietary phytoestrogens have protective effects against menopausal symptoms and a variety of disorders, including cardiovascular disease, cancer, hyperlipidemia, osteoporosis, and various forms of chronic renal disease (1-8). In this review, evidence for a possible role of dietary phytoestrogens in immunity is examined and various mechanisms by which this class of phytoestrogens may affect immunity are discussed.

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ISOFLAVONES

The majority of phytoestrogens found in typical human diets can be categorized into two primary classes : isoflavones and lignans. Phytoestrogens in the diet may have a role in modulating hormone-related disease based in their structural similarity to the estrogen 17β-estradiol (Fig. 1). Isoflavones make up the most common form of phytoestrogens. They have a common diphenolic structure that resembles the structure of the potent synthetic es-

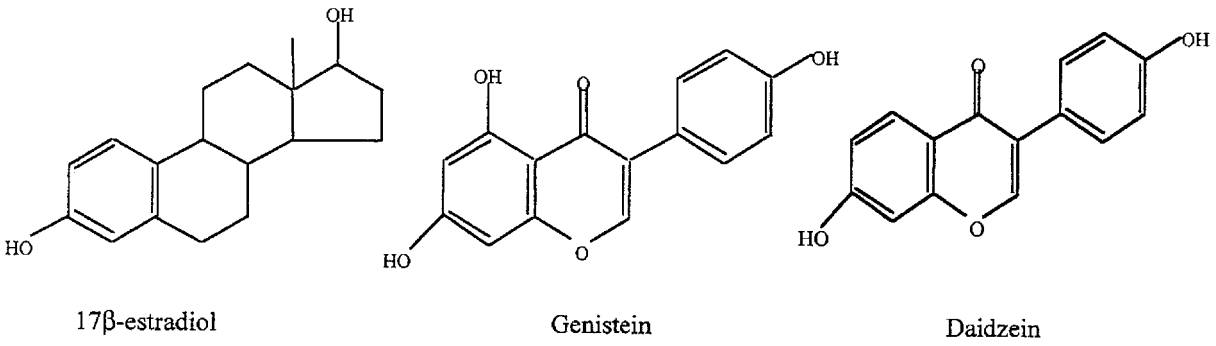

Fig. 1 Structure of soy isoflavones

trogens diethylstilbestrol and hexestrol. Two of the major isoflavones found in humans are genistein and daidzein. Genistein and daidzein are parent compounds, which are metabolized from their plant precursors, biochanin A and formononetin, respectively. In plants, isoflavones are inactive when present in the bound form as glycosides, but when the sugar residue is removed, these compounds become activated. These plant compounds undergo fermentation by intestinal microflora, with both metabolites and unfermented parent (aglycone) compounds being liable to absorption. In the body, they do not undergo any further metabolism and are excreted in the urine (9). In the colonic microflora, daidzein may be metabolized to equol or *O*-demethylangolesin and genistein may be metabolized to *p*-ethyl phenol. Daidzein, genistein, equol, and *O*-demethylangolesin are the major phytoestrogens detected in the blood and urine of humans and animals.

FOOD SOURCES OF PHYTOESTROGENS

Phytoestrogens are found in various plants consumed by humans, including legumes, seeds, and whole grains. The most abundant food sources of isoflavones are soybean and its products (Table 1).

Table 1. Isoflavone contents of soy products[1].

Soy products	Total isoflavones	Genistein	Daidzein
Roasted soybeans	2661[2]	1426	941
Soy-protein isolate	987	640	191
Tempeh	865	422	405
Tofu	532	245	238
Soy drink	28	21	7

[1] Adapted from Wang *et al.* (11).
[2] μg/g

Other beans, lentil, peas, and clover contain very small quantities of isoflavones. The amount of isoflavone in soybean varies according to the type of soybean, geographic area of cultivation, and harvest year. In addition, the isoflavone content of different soy products varies substantially as a result of differences in processing methods (10). In soybean, isoflavones are closely associated with protein. The protein content of soybeans is more than 36% by weight. Processed soybean proteins and foods provide various amounts of genistein and daidzein, as either conjugated glycones or as aglycone forms. Mature and roasted soybeans and commercially available soy products (soy flour and textured protein) contain 0.1-5 mg isoflavones/g protein. Green soybeans and tempeh are intermediate sources of isoflavones, providing 0.3 mg/g soy protein. One serving of traditional soy foods provides 0.25-40 mg isoflavones (11). Tofu, isolated soy protein, and some soymilk preparations provide 0.1-2 mg isoflavones/g soy protein. Alcohol extraction dissociates isoflavones bound to soy protein ; therefore, alcohol-denatured soy protein is devoid of a significant amount of isoflavones (12).

SOY ISOFLAVONE AND IMMUNITY

Genistein is one of the most extensively studied isoflavones for its effect on immunity. In some studies on the effect of genistein on immunity, ovariectomized (OVX) mice were used to avoid the effect of endogenous estrogen. Although this model is useful for investigating the direct effect of genistein on immune function, it does not always reflect physiological conditions *in vivo*. Indeed, some findings in OVX and non-OVX models are different, and care should be taken in interpreting those results (Table 2).

Table 2. Effects of soy isoflavones on immune functions *in vivo*

Species	Compound	Dose (day)	OVX	Effects	Reference
Mouse	Genistein	8-200 mg/kg	+	↓ thymus weight ↑ thymocyte apoptosis	14
				↓ number of peripheral lymphocytes	
				↓ Ag-specific Ab titer	
Mouse	Genistein	8-80 mg/kg	+	↓ DTH response	21
		1000-1500 ppm		↓ number of LN $CD4^+$ and $CD8^+$ T cells	
Mouse	Genistein	4-20 mg/kg	+	↓ Ag-specific T cell response	16
				↓ Ag-specific Ab titier	
				↓ Ag-specific cytokine production	
				→ Dendritic cell function	
				→ $CD4^+CD25^+$ T cell function	
Mouse	Genistein	30 mg/kg	-	↓ anti-collagen II Ab	15
				↓ DTH response	
Mouse	Genistein	4-20 mg/kg	-	↑ IFN-γ and IL-4 production	17
				↑ thymus weight	
Mouse	Genistein	2-20 mg/kg	-	↑ cytotoxic T cell and NK cell activity	19
				↑ resistance to B16F10 tumor	
Mouse	Genistein	4-20 mg/kg	-	↓ inflammatory dermatitis in NC mice	23
				↓ IFN-γ production ; ↑ IL-4 production	
Guinea pig	Genistein	15 mg/kg	-	↓ Ag-induced asthma	24
Mouse	Daidzein	10-40 mg/kg	-	↑ Thymus weight ; ↑ phagocytic activity	31
				↑ Ag-specific IgM Ab	

1) Lymphocyte proliferation response in vitro

A relatively high concentration of genistein inhibits lymphocyte proliferation response induced by mitogen or alloantigen *in vitro* (13). The tyrosine kinase signaling cascade plays a pivotal role in the activation of various inflammatory cells. Genistein is known to be an inhibitor of protein tyrosine kinase, and its activity may contribute to the suppressive effect *in vitro*.

2) Thymocyte differentiation

The thymus is a central organ for T cell differentiation. Genistein induces dose-responsive reductions in thymic weight and size in OVX mice (14). Genistein decreases thymocyte numbers by up to 86% and doubles apoptosis. Increased apoptosis is involved in the mechanism by which genistein causes loss of thymocyte. Administration of genistein to mice caused decreases in percentages of thymic $CD4^+CD8^-$ and double-positive $CD4^+CD8^+$ thymocytes, providing evidence that genistein may affect early thymocyte maturation and maturation of $CD4^+CD8^-$ helper T cells. Treatment of genistein-administered mice with anti-estrogen ICI 182,780 partially restored thymic weight. Therefore, the effect of genistein on thymic weight is mediated in part by the estrogen receptor.

3) Cellular and humoral immune responses

Genistein reduces the numbers of peripheral $CD4^+$ and $CD8^+$ T cells, and this reduction might come from thymic atrophy (14). Delayed-type hypersensitivity (DTH) reaction is classified as type IV allergy response and is mainly mediated by T cells and macrophages. Genistein suppresses DTH reaction to oxazolone and granulocyte-mediated response (15). In addition to cellular immune response, genistein also suppresses antigen (Ag)-induced antibody (Ab) production. In ovalbumin (OVA)-immunized mice, genistein suppresses OVA-specific IgG levels. Interestingly, an inhibitory effect of genistein on Ab production was not observed when thymus-independent Ag TNP-Ficoll was used (16), suggesting that the suppressive effect of genistein on Ag-specific Ab response is not a result of a direct inhibitory effect on B cells. In addition, genistein did not affect the expression of MHC class II, CD80 and CD86 and the Ag-presenting capacity of $CD11c^+$ dendritic cells (16). Although genistein inhibits OVA-specific T cell proliferation and cytokine responses, production of IFN-γ and IL-4

from T cells of genistein-treated mice is increased upon stimulation with anti-CD3 mAb (16, 17).

4) Tumor immunity

It has been reported that genistein increased host resistance to B16F10 tumor and induced a dose-dependent increase in cytotoxic T cell and NK cell activities (18, 19). However, genistein did not inhibit growth of tumor cells in athymic nude mice (20). These conflicting findings in euthymic and athymic mice suggest that genistein inhibits growth of a tumor not by direct inhibition but by enhancing immune cell function. The finding that tumor cells cultured with serum from genistein-treated mice did not suppress their growth ability supports the speculation that genistein enhances anti-tumor immunity (18).

5) Diseases (Animal model)

The effect of genistein on collagen-induced arthritis (CIA) has been investigated. Mice treated with genistein prior to immunization with collagen type II (CII) showed less frequent and less severe arthritis than did controls (21). Histopathological examination of the joints showed that synovial hyperplasia and bone/cartilage destruction was less frequent in joints of genistein-treated mice. An interesting finding is that levels of anti-CII-Abs in serum were significantly lower in groups of mice treated with genistein. Notably, there are significant correlations between CII-Ab levels and bone/cartilage destruction.

NC/Nga mice have been shown to develop spontaneous severe dermatitis when kept in conventional conditions (22). Oral administration of genistein suppresses the development of dermatitis but does not suppress serum IgE levels in NC/Nga mice. The mechanism underlying the suppressive effect of genistein on the development of dermatitis is not known, but little contribution of Th1/Th2 balance has been reported (23).

Allergic asthma is a chronic airway inflammatory disease that manifests itself as recurrent reversible acute bronchoconstriction and airway hyperresponsiveness (AHR). Duan, *et al.* examined anti-inflammatory effects of genistein on a guinea pig model of asthma (24). Genistein markedly inhibited OVA-induced and methacholin-induced acute bronchoconstriction. In addition, genistein reduced OVA-induced increases in total cell counts and eosinophils recovered in bronchoalveolar lavage fluid, and attenuated OVA-induced airway hyperresponsiveness to inhaled methacholine. The authors speculated that the inhibitory effect of genistein on AHR is attributed to the block of protein tyrosine kinase signaling cascades.

MECHANISMS OF THE EFFECTS OF GENISTEIN ON IMMUNE FUNCTIONS

Estrogen receptor-dependent and -independent mechanisms have been proposed for the immune modulating effect of genistein since genistein is structurally similar to estrogen. Indeed, expression of the estrogen receptor in thymocytes, lymphocytes and macrophages has been reported (25, 26). Estrogen is known to suppress the activity of immune cells and to suppress the development of DTH reaction (27), CII-induced arthritis (28) and experimental autoimmune encephalomyelitis (29) in animal models. It is possible that genistein has estrogen-like action and modulates immune function mediated by the estrogen receptor. However, several studies have shown that blockade of the estrogen receptor pathway partially abolishes the action of genistein. Genistein is known to be a broad-spectrum protein tyrosine kinase inhibitor (30), and its activity may contribute to one of the estrogen receptor-independent mechanisms. *In vitro* experiments have shown that genistein at a dose of more than 10 μM inhibits both tyrosine phosphorylation and binding of the nuclear factor to the specific promoter region, resulting in inhibition of proliferation response and cytokine production.

DAIDZEIN AND ITS METABOLITES

Data on the effects of formononetin, its metabolites daidzein and equol are limited. An *in vivo* study has shown that administration of daidzein increases the phagocytic response of peritoneal macrophages and the thymus weight in a dose-dependent manner (31), and it has been shown that daidzein increases proliferation response of splenocytes to both Con A and LPS stimulations *in vitro* (32). Formononetin and its metabolites have been found upregulate interleukin-4 production in activated T cells via increased AP-1 DNA binding activity (33). This finding suggests that phytoestrogen and some of their metabolites may affect allergic responses via the enhancement of IL-4 production in T cells.

PERSPECTIVE

Soy foods are traditionally consumed in relatively large amounts in Asian countries, such as China and Japan (34, 35), and in small amounts in Western countries, such as North American and European countries (36, 37). This may account for the lowered risk of hormone-related cancer and osteoporosis in Asian populations compared to that in Western populations (1-8). Recent evidence suggests that isoflavones in soy modulate immune function positively or negatively. The characteristic feature of genistein is its anti-inflammatory effect, and this effect has been demonstrated in animal models. However, epidemiologic study on the association of dietary soy or isoflavone consumption with allergic disorders is limited. Miyake, *et al.* conducted a cross-sectional study on the relationship between dietary soy products and isoflavone intake and the prevalence of allergic rhinitis (38). Compared with dietary intake of total soy product, soy protein, daidzein and genistein in the first quartile, consumption of these substances in the fourth quartile was found to be independently associated with reduced prevalence of allergic rhinitis, although no significant dose-response relationships were observed. This finding indicates the possibility that a high intake of soy and isoflavones is associated with reduced prevalence of allergic rhinitis. However, further investigations are needed to determine whether soy and soy isoflavone consumption has a preventive effect against allergic diseases.

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