Genistein Suppresses Development of Spontaneous Atopic-Like Dermatitis in NC/Nga Mice

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Summary In this study, we examined the effect of genistein on the severity of dermatitis and level of serum IgE in NC/Nga (NC) mice. NC mice housed in conventional conditions develop spontaneous atopic-like dermatitis; however, oral administration of 20 mg/kg genistein suppresses the development of dermatitis. We also investigated the levels of serum IgE in genistein-treated NC mice and found that the levels were the same as those in control NC mice. We further investigated in vitro IFN-γ and IL-4 production from spleen cells upon stimulation with anti-CD3 and anti-CD28 mAbs. IFN-γ production level in NC mice that received 20 mg/kg genistein was significantly lower than that in control NC mice. In contrast, the production level of IL-4 in genistein-treated mice was not significantly different from that in control mice but tended to increase in a dose-dependent manner.

Key Words genistein, NC/Nga mice, dermatitis, cytokine, IgE

The isoflavone genistein is a phytoestrogen found at high levels in soy products. Epidemiological studies, animal studies and in vitro experiments have indicated that genistein exerts beneficial effects in a multitude of disorders, including cancer (1, 2), cardiovascular diseases (3), osteoporosis, and postmenopausal symptoms (4).

Genistein has been shown to have an inhibitory effect on the activity of protein tyrosine kinase (5). This inhibitory effect leads to the suppression of lymphocyte function in vitro (6). In antigen (Ag)-immunized and genistein-treated mice, the magnitudes of delayed-type hypersensitivity reaction (DTH) and antibody (Ab) response were smaller than those in mice not treated with genistein (7, 8). In contrast to the immunosuppressive effect, administration of genistein significantly increases host resistance to a B16F10 tumor as reflected by a decrease in the number of lung tumor nodules after tumor cell injection. It has shown that enhancement of cytotoxic T cell and natural killer (NK) cell functions is responsible for this resistance (9). Enhancement of NK cell function has also been reported (10, 11). Therefore, genistein has biphasic effects on immune functions.

Atopic dermatitis (AD) is one of the most common skin diseases in children with a family history of atopy (12) and is frequently associated with elevated serum levels of IgE Abs against many kinds of inhaled allergens (13). NC/Nga (NC) mice have been shown to develop spontaneous severe dermatitis when kept in conventional conditions (14). The NC mouse has been used as a model animal for studies on allergies. In this study, we examined the effect of genistein on the severity of dermatitis and level of serum IgE in NC mice.

Materials and Methods

Animals and diet. Seven-week-old NC mice were purchased from SLC (Hamamatsu, Japan) and housed in conventional conditions. In this study, we used female mice only because male mice sometimes fought each other and this caused shin scars that influence the skin severity score.

The mice were provided with a soy isoflavone-free diet and water ad libitum. The composition of food was 20% casein, 44.7% α-starch (Oriental Yeast Co., Ltd., Chiba, Japan), 22.3% sucrose (Mitsui Sugar Co., Ltd., Osaka, Japan), 5% corn oil (Wako, Osaka, Japan), 2% cellulose, 5% mineral mixture and 1% vitamin mixture (Oriental Yeast Co., Ltd., Tokyo, Japan).

This study conformed to the guidelines for the care and use of laboratory animals of The University of Tokushima Graduate School Institution of Health Bioscience.

Genistein treatment. Genistein (LC Lab., MA, USA) solutions were freshly prepared daily in 25 mM Na2CO3. The mice were administered 200 μL genistein solutions containing 0 (25 mM Na2CO3), 4 or 20 mg genistein/kg body for 8 wk by gavage.

Dermatitis observation. At 0, 4 and 8 wk after the genistein treatment, the skin condition of each mouse was observed. Before skin conditions were scored, scratching behavior was observed for >2 min; itching...
was evaluated by observing scratching behavior. A total clinical severity score for atopic-like lesions was defined as the sum of individual scores graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for each sign and symptom (itch, erythema/hemorrhage and scaling/dryness).

**Cytokine production.** To prepare single cell suspensions, the spleen was squeezed with two slide glasses in RPMI-1640 medium (Sigma Chemical Co., MO, USA) and filtered through mesh. To remove red blood cells, the spleens were stimulated with plate-bound anti-CD3 mAb (eBio-science, CA, USA) and anti-CD28 mAb (eBioscience) (coated overnight at 2 μg/mL and 1 μg/mL respectively) in a 24-well flat-bottom plate at 37°C under 5% CO₂ for 10 min. Splenocytes (5×10⁶ cells/mL) were stimulated with plate-bound anti-CD3 mAb (eBio-science, CA, USA) and anti-CD28 mAb (eBioscience) (coated overnight at 2 μg/mL and 1 μg/mL respectively) in a 24-well flat-bottom plate at 37°C under 5% CO₂ for 48 h. After the culture, culture supernatants were collected and stored at −40°C until used. IFN-γ and IL-4 in the supernatants were determined using a Mouse IFN-γ or IL-4 ELISA kit (eBioscience) according to the manufacturer’s instructions.

**Determination of serum IgE level.** Blood collected from mouse tail with a capillary tube at 0, 4 and 8 wk after genistein treatment was centrifuged, and sera were stored at −20°C. Concentrations of serum IgE were determined using a Mouse IgE ELISA kit (Bethyl Laboratories, Inc., TX, USA).

**Statistical analysis.** Data were analyzed using one-way analysis of variance followed by the Scheffe post hoc test for multiple comparisons. Data are expressed as means±SD. Differences were considered significant at \( p<0.05 \).

**Results and Discussion**

NC mice kept in specific-pathogen free conditions remained healthy, but those kept in conventional conditions spontaneously developed a disease resembling human atopic-like dermatitis at the age of 6–7 wk (I4). In this study, NC mice were housed in conventional conditions at the age of 7 wk and treated with 4 mg/kg/d or 20 mg/kg/d of genistein every day for 8 wk. In the experimental period, oral administration of genistein did not significantly affect growth or final body weight (control group, 22.5±1.5 g; 4 mg/kg genistein group, 21.7±1.4 g; 20 mg/kg genistein group, 22.1±1.7 g). After 4 wk of genistein treatment, the severity of skin lesions in genistein-treated mice was not different from that in control mice. However, after 8 wk of treatment, the severity score of dermatitis in NC mice treated with 20 mg/kg genistein was significantly lower than that in control mice (Fig. 1A). The reduced severity score of dermatitis in the genistein-treated mice was mainly due to the decreased scratching behavior. We also measured serum IgE levels in those mice since in addition to development of dermatitis the level of total IgE in blood has been shown to increase in NC mice (I4). The level of total IgE in mice housed in conventional conditions was significantly higher than that in mice housed in specific-pathogen free conditions. A significant difference was not found between the serum levels of IgE in mice treated with genistein and mice not treated with genistein (Fig. 1B). These results suggest that genistein suppresses the development of dermatitis but does not affect IgE production in NC mice.

Elevation of total IgE has been reported to correlate with the appearance of atopic dermatitis-like lesions in NC mice, with massive infiltration of CD4⁺ T cells producing IL-4 and IL-5, and with degranulation of mast cells and eosinophils (I4). These pathophysiological observations in dermatitis of NC mice resemble those in human AD. In NC mice, constitutive tyrosine phosphorylation of Janus kinase 3, a tyrosine kinase responsible for IL-4R-mediated signaling, is thought to be involved in the enhanced sensitivity of B cells to IL-4, leading to the elevation of total IgE levels (I5). We assessed T helper type 1 (Th1)/Th2 responses in genistein-treated NC mice to determine the contribution of these subsets to the development of dermatitis. IFN-γ production level in NC mice that received 20 mg/kg genistein was significantly lower than that in control NC mice (Fig. 2A). In contrast to IFN-γ production level, production level of IL-4 in genistein-treated mice was not significantly different from that in control mice but tended to increase in a dose-dependent manner (Fig. 2B). These results
sugest that the suppressive effect of genistein on the development of dermatitis is independent of IL-4, although IL-4 has been shown to contribute to many allergic responses (16). It has been reported that there was little contribution of IL-4 to the development of dermatitis in NC mice (17). Signal transducer and activator of transcription 6 (STAT6) is a critical transcriptional factor that regulates IL-4-mediated immune responses. STAT6 is phosphorylated and activated through an IL-4R-mediated signal; it translocates as a phosphorylated homodimer and subsequently regulates IL-4-mediated transcriptional events, including Th2 differentiation, expression of a cell surface marker, and Ig class switching to IgG1 and IgE (18). Yagi et al. generated STAT6-deficient NC mice and investigated the development of dermatitis in those mice. The frequency and severity of dermatitis in STAT6-deficient NC mice were not different from those in NC mice, while elevation of serum IgE level was not observed (17).

Genistein displays structural similarity with 17-β-estradiol and has been shown to have estrogenic or anti-estrogenic properties. Phytoestrogens, including genistein, exhibit weak estrogenic activity in the order of $10^{-3}$ to $10^{-4}$ that of 17-β-estradiol (19) but may be present in the body at concentrations 22,000-fold higher than concentrations of endogenous estrogens in soy formula-fed infants (20). The anti-estrogenic activity of genistein may be partially explained by the competition of genistein with endogenous 17-β-estradiol for the estrogen receptor (ER). 17-β-Estradiol has been shown to preferentially induce IFN-γ-producing cells (21, 22). Th1 and Th2 cells are reciprocally regulated by each subset. Therefore, one of mechanisms by which IFN-γ production is suppressed and IL-4 production is increased might be competition of genistein with endogenous 17-β-estradiol for the ER.

The exact mechanism of the suppressive effect of genistein on development of dermatitis is currently unknown. Genistein is speculated to exert its function through ER-dependent and ER-independent mechanisms. Indeed, genistein has been shown to suppress DTH and Ag-specific Ab production in partially ER-independent manners (7). A property of the protein tyrosine kinase inhibitor is thought to be major ER-independent action by genistein. Cumulative evidence concurs with the fact that the tyrosine kinase signaling cascade plays a pivotal role in the initiation of activation of various inflammatory cells that are important for the pathogenesis of allergic inflammation (23). In an in vitro experiment, genistein was found to inhibit lymphocyte proliferation response in a dose-dependent manner (6). Furthermore, it has been reported that genistein exerts tyrosine kinase inhibitory activity and suppresses airway hyperresponsiveness in a guinea pig model of asthma (24).

In conclusion, we found in this study that genistein suppresses the development of atopic-like skin lesions in NC mice. To our knowledge, this is the first study to show a therapeutic effect of genistein on dermatitis in a spontaneous animal model.

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