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Modulation of immune response following dietary genistein exposure in F_0 and F_1 generations of C57BL/6 mice: Evidence of thymic regulation

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Abstract

To further determine whether genistein (GEN) modulation of the immune responses was related to its endocrine-disrupting properties and time of exposure, pregnant C57BL/6 mice were exposed to GEN at 0–1250 ppm in feed starting on day 14 of gestation. The C57BL/6 offspring were exposed to GEN in utero and lactationally, and through feed after weaning until postnatal day 42. In dams, exposure to GEN increased the terminal body weight (250 and 1250 ppm), the number of splenic T cells and NK cells (250 ppm), and the activity of NK cells (250 ppm). In F_1 males, GEN increased the terminal body and spleen weights (25 and 250 ppm), the number of $CD4^+CD8^+$ and $CD4^-CD8^+$ thymocytes (25 ppm), and the number of splenic T cell subsets and NK cells (25 and 250 ppm). Moreover, splenic NK cell activity and anti-CD3-mediated splenocyte proliferation were increased in all treatment groups. In F_1 females, the percentages of $CD4^-CD8^+$ and $CD4^-CD8^-$ thymocytes (25 and 250 ppm), and $CD4^+CD8^-$ and $CD4^+CD8^+$ splenocytes (25 and 250 ppm) were increased. In contrast, the percentage and number of $CD4^+CD8^+$ thymocytes were decreased (250 ppm). Exposure to GEN decreased the percentages of splenic NK cells in all treatment groups, and decreased the activity of splenic NK cells at the 25 ppm concentration. Additionally, evaluation of $CD25^+$ and $CD44^+$ expression by thymocytes indicated that the decrease in the percentage of $CD44^+CD25^+$ thymocytes was at least partially responsible for the decrease in the percentage of $CD4^-CD8^-$ thymocytes in F_1 male mice. Overall, the results demonstrate that GEN can modulate the immune system in both adult and developing C57BL/6 mice in a dose-specific manner. The gender-specific effects of GEN on the immune responses in F_1 mice suggest that GEN may modulate the immune system by functioning as either an estrogen agonist or antagonist. The differential effects of GEN on thymocytes in F_1 male and female mice indicate that GEN immunomodulation might be related to its effect on thymus.

Keywords: Genistein; Natural killer cell activity; Anti-CD3 mediated proliferation and phenotypic markers

1. Introduction

Genistein (GEN), a major isoflavone in most soy products, has been demonstrated to interact with estrogen receptors (ERs) in vivo (Martin et al., 1978). It has also been utilized as a tyrosine kinase inhibitor in vitro (Kurzer and Xu, 1997). Despite the hypothesized

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beneficial effects of GEN, e.g., decreased incidences of some hormone-related cancers (Bingham et al., 1998), there are concerns about the long-term effects of this compound on human health, especially that of infants and young children. Infants fed soymilk formulas have plasma isoflavone levels that are orders of magnitude higher than those of infants fed human or cows' milk (Setchell et al., 1997). The possible long-term effects of these relatively high levels of phytoestrogens during infancy are unknown. A retrospective multiple controlled cohort study indicated that there was an increase in the use of asthma or allergy drugs in individuals who were fed soy formula during infancy as compared to those who were fed cow milk formula (Strom et al., 2001).

Our previous studies have demonstrated that exposure to GEN (2–20 mg/kg) in adult female B6C3F1 mice for 28 days by gavage increased the activities of cytotoxic T cells and natural killer (NK) cells (Guo et al., 2001). Additionally, increased splenic T cell number was observed in male and female Sprague Dawley rats when the rats were exposed to GEN-containing feed (25–1250 ppm) gestationally, lactationally and from feeding from gestation day (GD) 7 to postnatal day (PND) 64 (Guo et al., 2002a). However, differential effects on NK cell activity were observed in male and female Sprague Dawley rats when the rats were exposed to GEN-containing feed (300–800 ppm) gestationally and lactationally from GD 1 to PND 21 (Guo et al., 2002b). Importantly, both natural killer (NK) and T cells have been demonstrated to contribute significantly to the disease's persistence and progression in asthma and allergy (Korsgren et al., 1999; Cohn et al., 2004).

There is evidence that the time of exposure to GEN predetermines GEN's biological effects (Lamartiniere et al., 2002). The most susceptible period for human exposure to estrogenic compounds has been reported to be from the last trimester of pregnancy to 18 years of age, which is approximately corresponding to the period of GD 14 to PND 42 in mice (Chapin et al., 1997). Estrogen active chemicals may function as either a pro- or an anti-estrogen in different estrogenic environments (Bouker and Hilakivi-Clarke, 2000). Thus, it was hypothesized that the activation of T cells and NK cells could be differentially modulated by GEN in C57BL/6 mice during adult exposure in dams from day 14 of pregnancy to postpartum day 42 and during developmental exposures in male and female offspring from GD 14 to PND 42. In this study, we have evaluated the effects of GEN on the activities of NK cells and T cells in both F_0 and F_1 generations of C57BL/6 mice by administering GEN-containing food (0–1250 ppm) to the animals. Thymus has been demonstrated to be the major organ where estrogen exerts its immunomodulatory effects (Luster et al., 1984; Erbach and Bahr, 1991); therefore, the effects of GEN on the phenotypic marker expression by thymocytes were evaluated in F_1 male and female C57BL/6 mice.

2. Materials and methods

2.1. Animals and treatments

Time pregnant young adult C57BL/6 mice were purchased from Charles River Breeding Laboratories (Raleigh, NC) and delivered to our animal facility on GD 14 (plug date = GD 0). The pregnant mice were housed individually in standard plastic cages with hardwood chip bedding. The animal room was maintained within a temperature range of 22–25 °C and relative humidity of 50 ± 10 with 12-h light cycles (7:00–19:00). The mice were randomized into different treatment groups (4–5 mice per dose group) on the day of delivery, and each group was provided immediately with one of the treatment diets described below and water ad libitum. After parturition, the offspring were housed together with their respective dams, one litter per cage, until weaning on PND 22, at which time the offspring were housed up to four same-sex littermates per cage. To eliminate litter effect and, at the same time, to maintain appropriate number of mice in each group for statistical analysis, at least one but not more than two mice from each litter for each sex were randomly selected for evaluation.

The diet (5K96, purchased from Purina Mills, St. Louis, MO) is based on the NIH-31 formula, except that casein replaces the protein contributed by soy and alfalfa, soy oil is replaced by corn oil, and the vitamin mix is adjusted for irradiation. The control diet was assayed for genistein and daidzein after hydrolysis of conjugates. The concentrations of both genistein and daidzein of this diet were determined by LC–ES/MS/MS to be approximately 0.5 ppm (Doerge et al., 2000). The dams consumed 5K96 chow containing 0–1250 ppm GEN (Toronto Research Chemicals, North York, Ontario, Canada) for 50 days starting on day 14 of gestation. The F_1 mice were exposed to GEN gestationally and lactationally, and to GEN-containing feed from the day of weaning (PND22) to PND42. GEN with purity greater than 99% was mixed into the standard 5K96 feed every three months by the Diet Preparation Staff, Bionetics at the National Center for Toxicological Research (NCTR, Jefferson, AR); each dosed batch of feed was analyzed by the Division of Chemistry, and it was stable for at least six months when stored refrigerated.

On PND 42, dams and pups were sacrificed by CO_2 inhalation, and the spleens and thymuses were collected for immunological evaluations. All animal procedures were conducted under an animal protocol approved by the VCU Institutional Animal Care and Use Committee (IACUC).