Genistein, Estrogen Receptors, and the Acquired Immune Response\textsuperscript{1,2}

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**ABSTRACT** Estrogen regulates thymic development and immune function. Despite the critical role of estrogens in inducing thymic involution and modulating immune responses, the mechanism of this effect is unclear. Similarly, humans and animals are exposed to increasing amounts of the estrogenic soy isoflavone genistein in the diet, but whether genistein can induce immune changes has not been definitively established. We reported previously that genistein induces thymic atrophy in mice, and decreases both humoral and cell-mediated immunity. These thymic effects of genistein occur via estrogen receptor (ER)-mediated and non-ER–mediated pathways. Genistein injections produced the most pronounced effects, but dietary administration to mice that produced serum genistein concentrations similar to those reported in human infants consuming soy formula also had demonstrable effects. Microarray analysis of the effects of estradiol and genistein on neonatal thymus indicated that estradiol affected genes involved in transcription, apoptosis, cell cycle, and thymic development and function; genistein had similar effects on many estradiol target genes, but also had unique actions not replicated by estradiol. Despite extensive work showing inhibitory effects of genistein on immunity, other rodent studies reported that genistein or other phytoestrogens stimulate various aspects of immune function. Although the present data strongly indicate that genistein can regulate immune function, possibly at physiologic concentrations, further work is required to definitively establish overall thymic and immune effects of genistein and soy, which may vary with age, species, and specific end point. J. Nutr. 136: 704–708, 2006.

**KEY WORDS:** immunity • phytoestrogens • isoflavones • soy • thymus

The possible effect on humans and animals of environmental chemicals that either mimic or inhibit the actions of endogenous hormones has become an area of great concern for both the scientific community and general public. Phytoestrogens represent a large group of estrogens derived from plant sources. These chemicals are ubiquitous in our food supply and these compounds may affect a variety of biological processes that have relevance for human and animal health. The immune system is a major estrogen target, and recent data indicated that one group of phytoestrogens, the isoflavones, can produce immune effects, although these studies have been inconsistent or even conflicting at times. Here, we summarize current knowledge related to the potential effects of isoflavones on the immune system.

**Human exposure to estrogenic soy isoflavones.** Soybeans and foods containing soy have significant levels of isoflavones such as genistein and daidzein. Genistein, daidzein, and the daidzein metabolite equol are structurally similar to 17β-estradiol (E2)\textsuperscript{4} and have estrogenic effects. Soy phytoestrogens bind to both the classical estrogen receptor, estrogen receptor-α, (ER\textsubscript{α}, and the more recently discovered second form of ER, ERβ). In contrast to the most potent endogenous estrogen, E2, which binds ER\textsubscript{α} and ERβ with similar affinities, genistein, daidzein, and equol are unique in that they have greater affinity for ERβ than ER\textsubscript{α}. Despite the limited estrogenic potency of these compounds, their high levels in humans under certain nutritional conditions could induce significant biological effects.

In populations that have historically consumed large amounts of dietary soy, such as the Japanese, adult men take in up to 1 mg of isoflavones/[kg body weight (BW)]-d and have relatively high levels of serum isoflavones (0.16–0.89 μmol/L; mean = 0.38 μmol/L), a concentration several times that of

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\textsuperscript{4} Abbreviations used: BW, body weight; E2, 17β-estradiol; ER, estrogen receptor; NK, natural killer.
men consuming typical Western diets containing minimal soy (1). In contrast to the exposure of humans to dietary soy, which has occurred for centuries without obvious deleterious effects, soy and isoflavone supplements have become increasingly popular in recent years. These products, consumed predominantly but not exclusively by postmenopausal women, can produce greater exposure to isoflavones than could be obtained even with consumption of a high-soy diet. These supplements represent an increasing source of high-level isoflavone exposure that has been considered safe based on the long history of dietary consumption of soy by humans; however, the potentially greater levels of exposure obtained with supplements raises the possibility of effects at these levels, both good and bad, that are not seen with the consumption of more modest levels as one constituent of a normal diet.

A similar argument can be made for infants consuming soy-based infant formula. The present soy-based infant formulas were developed in the 1960s, and have become increasingly popular in recent years, especially in certain Western countries such as the United States. Of formula-fed infants in the United States, ~25% (~750,000 infants/yr) consume soy-based formula (2). It is estimated that ~20,000,000 infants in the United States have consumed soy-based infant formula since these products became widely used (2). There is additional exposure in other parts of the world, although these products are less extensively utilized in other countries such as the U.K. or Australia, and they are not used extensively in many parts of the world.

Consumption of soy-based infant formula results in high genistein and daidzein exposure during a developmental period when humans have low endogenous estrogen levels and have historically been exposed to low amounts of isoflavones. Because this is initially their sole nutritional source, infants consume 6.0–11.9 mg of isoflavones/(kg BW·d) (3), an order of magnitude greater than adults eating a high-soy diet.

Human infants efficiently digest and absorb genistein and daidzein (4), and have plasma levels of isoflavones from 2.0 to 6.6 μmol/L, with a mean of 3.7 μmol/L (3). This level is 10-fold greater than that of adults consuming high-soy diets (0.19–0.75 μmol/L) and Japanese adults (mean = 0.38 μmol/L) whose diets have historically included soy. Although limited studies on adults exposed to soy formula during infancy have not documented any reproductive abnormalities in this population (2), soy-fed human infants are exposed to chronic isoflavone levels much greater than those in adults, and the long-term biological effects of this high level of exposure, if any, are unclear.

**Immune effects of genistein and other phytoestrogens.** Soy phytoestrogens have reproductive effects in laboratory animals and humans (5). However, ER are also widely distributed in nonreproductive tissues, and these organs are also estrogen targets. Immune tissues and cells express ER and respond to estrogens. For example, thymus expresses both ERα and ERβ and normal thymic development is dependent on the E2/ERα signaling pathway, as shown by reduced thymic size in neonatal mice lacking ERα (6,7).

Despite the involvement of E2/ERα signaling in normal thymic development, administration of exogenous estrogen to pubertal or adult animals induces thymic atrophy and immune suppression (8). However, early estrogen exposure during the perinatal period can induce either decreases or increases in thymic weight, and potentially, functionality; this is variable depending upon the age of the animal and dose.

An extensive literature has documented inhibitory effects of genistein on various aspects of immune cell function in vitro, but this must be interpreted with care. High concentrations of genistein are an effective protein tyrosine kinase inhibitor, and genistein is routinely used at clearly pharmacologic concentrations as a biochemical tool to inhibit these enzymes. Because these enzymes are involved in many signaling pathways in various types of immune cells, it is not surprising that there are many reports of inhibitory effects of genistein at high concentrations on immune cells (9). However, genistein inhibits tyrosine kinases only at supraphysiologic concentrations (typically 100 μmol/L), which are manyfold greater than concentrations that can be obtained through consumption of dietary genistein or even through the use of supplements containing genistein. Therefore, these types of studies (9) typically do not seek to infer any possible effects of physiologic concentrations of genistein on human or animal immune cells and will not be considered here.

There are a number of other reports in which genistein treatment of immune cells was used as a means of potentially gaining insight into the effects of exposure to genistein on human and animal immune cells. Genistein decreases nitric oxide production in cultured mouse macrophages, suggesting that it could modulate immune responses. In addition, genistein at high doses inhibited the proliferation of cultured T cells in response to CD28 monoclonal antibody, and also inhibited production of interleukin Tα, interleukins, and the interleukin receptor [reviewed in (10)]. Genistein at high concentrations in vitro also inhibits cytotoxic T-cell–mediated tumoricidal activity, alters leukocyte adherence, impairs T-cell motility and inhibits the activation of natural killer (NK) cells in response to lipopolysaccharide or fixed bacteria [reviewed in (10)]. However, studies showing genistein effects on immune cells or functions in vitro have typically used genistein concentrations higher than would be obtained in vivo; thus, it is unclear whether similar effects occur at physiologic concentrations of genistein in vivo. For example, genistein at 1–100 μmol/L inhibited the growth of human leukemic mast cells, and at 100 μmol/L, it altered the histamine content of these cells (11). Although human concentrations of genistein ≥ 1 μmol/L have been reported, the higher concentrations of genistein (10–100 μmol/L) used in these studies are difficult to produce in vivo for sustained periods of time, and are clearly not physiologic. Therefore, many in vitro results cannot be used reliably to gain valid insights into the effects of physiologic concentrations of genistein in vivo. However, in some cases, the lower doses used in these studies extended down into the range reported for humans after various types of genistein exposure in vivo, and determination of whether a particular in vitro study has relevance for the in vivo situation must always take into account the genistein concentrations used in vitro relative to those in vivo.

A number of recent studies addressed the potential effects of genistein in vivo on the immune system, especially the thymus. The thymus is largest during fetal and early postnatal life, and developmental toxicological insults have the greatest potential to depress immune function and/or increase susceptibility to autoimmune disease (8). We reported that genistein injected into ovariectomized juvenile mice led to dose-responsive decreases in thymic weight up to 80% (Table 1) (12). Both the route of administration and potentially the serum genistein concentrations produced by injection are not physiologic; thus, these results clearly do not demonstrate a risk from dietary genistein consumption, but they do underline the ability of genistein to induce thymic changes. In addition, dietary genistein in amounts that produced serum genistein concentrations that can be obtained in humans also decreased thymic weight, although the observed changes were far less than those seen with injections (12). Genistein caused its effects through both ER- and non-ER–mediated pathways. Genistein injection
reduced thymocyte numbers and increased apoptosis. The effect of genistein was selective in that CD4^+CD8^- single positive cells and CD4^-CD8^+ double positive cells were preferentially decreased in the thymus. The decrease in the CD4^-CD8^- thymocytes was accompanied by a significant decrease in the splenic CD4^-CD8^- percentages and a systemic lymphocytopenia. In addition to the changes in the cellular composition of lymphoid organs, genistein produced a dose-dependent suppression of both humoral as well as cell-mediated immune responses (Table 1) (12,13). Interestingly, the effects of genistein on the thymus and the immune system were reversible, and both thymic size and immune response returned to normal after cessation of the genistein treatments. In conclusion, our studies showed that genistein can produce thymic and immune changes in mice. Genistein injections typically produced the greatest effects, but effects were also detectable when genistein was given in the diet at levels that produced serum genistein concentrations that can be obtained in humans, raising the concern that high levels of genistein exposure in humans may at least have the potential to produce immune changes.

There are numerous other reports of inhibitory genistein effects on aspects of immune function that have been derived from a wide array of experimental approaches. Curran et al. (14) reported that dietary genistein or soy could inhibit the amount of IFN-\(\gamma\) normally produced in response to a bacterial infection in mice (Table 1). This study is especially noteworthy in that these effects were obtained with dietary genistein or soy supplementation that produced circulating serum genistein concentrations of \(\sim 0.4\) and \(\sim 1\) \(\mu M/L\); the former concentration is comparable to serum genistein concentrations reported in Japanese men consuming normal diets containing soy.

Although work with both estrogens and phytoestrogens has focused on developmental exposure to these chemicals to alter immune function, phytoestrogens may also have significant immune effects in aged animals. Calemine et al. (15) gave geriatric mice oral genistein (1.5 mg/kg) for 2.5 wk, and found that IFN-\(\gamma\) production by splenocytes was decreased in genistein-treated compared with the control mice (Table 1). Potential immune inhibitory effects of genistein or other phytoestrogens would normally be considered deleterious, but these inhibitory effects may be beneficial or desirable in certain situations (e.g., autoimmune diseases or after organ grafting). For example, O’Connor et al. (16) showed that a high-isoflavone diet or i.v. genistein produced immune inhibition, as reflected by delayed rejection of cardiac allografts in rats (Table 1). The effects of genistein on organ rejection were also additive with those produced by cyclosporine, the drug used for suppression rejection in transplant recipients. Similar results were obtained by Regal et al. (17), who showed that a diet containing high levels of genistein had a beneficial anti-inflammatory effect in a guinea pig model of asthma, in which it reduced antigen-induced eosinophilia in response to an antigen challenge (Table 1). Thus, the immune suppression produced by genistein can be beneficial under certain conditions, and has at least the potential to be harnessed clinically.

In contrast to the literature showing suppression of various immune parameters after genistein exposure in mice, Guo et al. (18) reported that genistein increased host resistance to the B16F10 tumor in adult female mice, and induced a dose-dependent increase in cytotoxic T cell and NK cell activity (Table 1). This group (19,20) later showed that the effects of genistein in rats varied with age (Table 1), as well as sex, with immune effects in one sex not seen in the other sex given similar genistein exposure. Klein et al. (21) also suggested a stimulatory effect of genistein on the thymus, reporting that perinatal exposure of rats to genistein resulted in increased thymic size in the adult rats and also caused changes in the percentage of certain types of thymocytes and splenocytes (Table 1).

Data on the effects of daidzein and its metabolite equol are limited. Previous reports using both in vitro (22) and in vivo (23) approaches suggested that daidzein may be immunostimulatory. In contrast, studies with equol did not report an effect on thymic weight with physiologic concentrations in vivo (24), suggesting that equol may not be capable of producing the thymolytic effects seen with genistein.

Although there is a paucity of information regarding human immune effects of soy isoflavones, there have been some studies...
in both human adults and infants. Administration of a high-soy diet containing 73 mg/d of isoflavones increased IL-6 concentrations in women; a similar effect was not seen in men, and the low-soy diet (10 mg/d of isoflavones) did not have a similar effect on IL-6 (25). The literature on the effects of soy-based infant formula is directly contradictory, with older work from Italy indicating that infants fed soy flour–based formula had increased morbidity and decreased responses to vaccines, whereas more extensive newer work from the manufacturers of the presently used soy protein isolate–based formulas did not find these types of immune impairments [reviewed in (26)].

Effects of genistein on thymic gene expression. To provide insights into the mechanisms of E2 and genistein effects in the thymus, we investigated thymic gene expression changes induced by E2 and genistein in ovariectomized weaning mice using high-density DNA arrays (27). We identified several E2 responsive genes involved in thymic development and thymocyte signaling during selection and maturation (gene expression data are available from the GEO database http://www.ncbi.nlm.nih.gov/geo/ with the accession number GSE2889).

Functional characterization indicated effects on genes involved in transcription, apoptosis, and the cell cycle. This study also identified changes in several E2-regulated transcripts essential to maintain immune self-tolerance. E2 upregulated more genes than genistein, whereas genistein downregulated more genes than E2. Although each treatment regulated several genes not altered by the other, there was substantial overlap in the genes regulated by E2 and genistein. Changes in transcription factors and cell cycle factors were consistent with decreases in cell proliferation induced by both genistein and E2. As indicated by the regulation of non-E2–responsive genes, genistein also induced unique effects through nonestrogenic mechanisms. The specific downregulation of the CD4+ thymocyte transcript by genistein was consistent with the decline of CD4+ thymocytes in genistein-treated mice in our previous study. This study identifying E2 and genistein target genes in the thymus may provide new mechanistic insights toward explaining estrogen action on thymocyte development, selection, and maturation, as well as the effects of genistein on thymic development and function.

SUMMARY AND CONCLUSIONS

Work from a number of laboratories indicates that genistein and other phytoestrogens can cause immune effects when injected as well as when given in a more physiologically relevant manner such as in the diet or by gavage. However, the current literature is incomplete and sometimes contradictory. The exact role phytoestrogens could play in terms of modulating immune activity in humans therefore remains unclear, but extensive animal work showing isoflavone effects on immune parameters suggests that immune effects of genistein and daidzin in humans are feasible. Previous work focused heavily on genistein, but soy contains many bioactive compounds in addition to genistein (28). Therefore, additional study is warranted to develop a more complete understanding of the effects of soy on specific immune cells and the immune system in general because the effects of genistein on an immune parameter would not necessarily correspond to the effects that might be produced by exposure to the more complex soy products that people actually consume (28). A simplistic, generalized conclusion concerning whether phytoestrogens and soy have beneficial or detrimental immune effects likely will not be possible; instead, a consideration of whether a particular exposure could have positive or negative will likely depend on the species, sex, level of exposure and dosing regimen, age at exposure, and the particular end point being examined.

LITERATURE CITED


