Soy Isoflavone Aglycone Modulates Expression of Cell Surface Antigens in Vitro and in Vivo

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Soy isoflavone aglycones (IFAs) have a wide range of biological actions. We investigated in this study the effect of IFAs on myeloid cells. The cell surface expression of both CD80 and CD86 was up-regulated by treating myeloid cells with IFAs in vitro and in vivo. The findings suggest that IFAs could modulate the myeloid cell function.

Key words: soy isoflavone aglycone; myeloid cell; CD80; CD86

Epidemiological evidence and experimental data from animal studies strongly support the beneficial effects of isoflavones on human health.1) In mammalian systems, soy isoflavones exhibit a number of biological activities, including the inhibition of cell proliferation,2,3) antioxidative effects,4) and enzyme-inhibitory effects.5) In addition, dietary soy isoflavones have been considered for the treatment and prevention of hormone-dependent diseases.6)

Both lymphoid and myeloid cells express estrogen receptors, and the steroid sex hormone, estrogen, has been demonstrated to influence immune cells as a growth and differentiation factor with effects on hemopoiesis, lymphocyte activation, Th polarization, and cytokine production.7) Despite the critical role of estrogens in inducing modulating immune responses, the mechanism for this effect is unclear. Genistein and daidzein, which are the best known isoflavones, are structurally similar to 17β-estradiol (E2) and have estrogenic effects. Genistein or other phytoestrogens stimulate various aspects of the immune function.8) These findings suggest that isoflavones could modulate immune responses. We prepared soy isoflavone aglycones (IFAs) and have been studying their biological activities.9,10) A fermented soybean extract (FSBE) was provided by Kikkoman Co. (Chiba, Japan) as a yellow-brown powder. FSBE, which is rich in the two major isoflavone aglycones, genistein and daidzein, was used as the source of IFA. The composition of FSBE was 341 mg/g of isoflavone as the aglycone form (193 mg/g of genistein, 148 mg/g of daidzein and a trace of glycitein), 183 mg/g of protein, 258 mg/g of carbohydrate, 116 mg/g of fat, 16 mg/g of moisture, 24 mg/g of ash and 62 mg/g of fiber. We investigated in this study the effect of IFAs on myeloid cell activity in vitro and in vivo.

To effectively stimulate naive T cells, antigen-presenting cells undergo maturation in the presence of inflammatory stimuli or products derived from pathogens, a process characterized by marked up-regulation of cell surface peptide-loaded MHC proteins and such costimulatory molecules as CD80, CD86, and CD40.11) To examine the effect of IFA on myeloid cell activity, we focused on the expression of CD80 and CD86 on myeloid cells in vitro. The murine macrophage cell line, J774A.1 (BALB/c, macrophage), was cultured in the presence of IFA (0, 1 or 50 μg/ml) for 24 h. Flow cytometry was used to evaluate the cell surface expression of CD80 and CD86 on the J774A.1 cells. FITC-anti-mouse CD80 (BD Biosciences) and PE-anti-mouse CD86 (BD Biosciences) were used for staining the J774A.1 cells. Figure 1 shows the results for the expression of CD80 and CD86, both being up-regulated by IFA. Of these costimulatory molecules, CD80 showed a more significant enhancement of expression than CD86. These results suggest that IFA could modulate the myeloid cell function, as assessed by the level of such activation markers as CD80 and CD86, in vitro.

To identify the substance responsible for the observed effect of IFA, we measured the effects of genistein and daidzein on the expression of cell surface...
**Fig. 1.** Expression of CD80 and CD86 on J774A.1 Cells Stimulated with IFA.

J774A.1 cells, a murine macrophage tumor cell line, were maintained in RPMI 1640 supplemented with 10% FBS (Gibco BRL, Gaithersburgs, MD, USA), 25 mM Hepes, 0.1 mM 2-mercaptoethanol, 100 U/ml of penicillin, and 100 µg/ml of streptomycin. The cells (2.5 × 10^5 cells/ml) were incubated with IFA at the concentration of either 50 or 1 µg/ml dissolved in DMSO in a 24-well tissue culture plate for 24 h. The cells were then stained for (a) CD80 and (b) CD86, and analyzed by flow cytometry. Black lines, non-stained negative control; filled histograms, stained positive control (basal level of expression); green line, same vol. of DMSO to dissolve 50 µg/ml of sample added; red lines, same vol. of DMSO to dissolve 1 µg/ml of sample added.

**Fig. 2.** Expression of CD80 and CD86 on J774A.1 Cells Stimulated with Genistein or Daidzein.

J774A.1 cells were maintained in RPMI 1640 supplemented with 10% FBS, 25 mM Hepes, 0.1 mM 2-mercaptoethanol, 100 U/ml of penicillin, and 100 µg/ml of streptomycin. The cells (2.5 × 10^5 cells/ml) were incubated with genistein or daidzein at the concentration of either 50 or 1 µg/ml dissolved in DMSO in a 24-well tissue culture plate for 24 h. The cells were then stained for (a) CD80 and (b) CD86, and analyzed by flow cytometry. Black lines, non-stained negative control; filled histograms, stained positive control (basal level of expression); green lines, 50 µg/ml of sample added; red lines, 1 µg/ml of sample added.
molecules. The cell surface expression of CD80 and CD86 on J774A.1 cells was analyzed by flow cytometry after incubating cells with genistein or daidzein (1 or 50 μg/ml). Figure 2 shows that genistein, but not daidzein, induced the expression of CD80 and CD86. In addition, we analyzed whether treating J774A.1 cells with IFA, genistein, or daidzein would modulate the expression of the cell surface molecules, Ia and ICAM-1, both of which are required for the antigen-presenting function. However, neither of these molecules was affected by isoflavone (data not shown). It is thus suggested that genistein was responsible for the up-regulation of both CD80 and CD86.

Next, we examined the effect of IFA on myeloid cells in vivo. Female DBA/2 mice (Japan SLC, Shizuoka, Japan) were orally administered with IFA (0, 15, 30 or 60 mg/mouse) for 3 days twice every day. After this administration, PECs were removed from these mice. These cells were stained for CD11b and CD80 or CD86, and then analyzed by flow cytometry. Aa, data show CD11b expression; b, data show CD80 expression on CD11b-positive cells from IFA-15 mg/mouse treated mice. B, data show the mean fluorescence intensity (MFI) of CD80 (a) and CD86 (b) expression on CD11b-positive cells. The data show one of two experiments performed with similar results, each evaluating two mice per group. Each value represents the mean ± SD. Significant difference from the control, *p < 0.05.

Work from a number of laboratories indicates that isoflavones can cause immune effects when injected as well as when given in a more physiologically relevant manner such as in the diet. We have demonstrated in this study that IFAs could modulate the myeloid cell function, as assessed by the level of such activation markers as CD80 and CD86, in vitro and in vivo. We have also shown that genistein, but not daidzein enhanced the expression of CD80 and CD86 (Fig. 2). Genistein, the main isoflavone present in soy, has been reported to have significant protective effects against induced and spontaneous cancers. Genistein has inhibited the production of leukotriene B4, interleukins, and the interleukin receptor.12) These inhibitory effects may be beneficial or desirable in such situations as autoimmune diseases. In contrast, Guo et al.13) have 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. We have also previously reported that the hematopoietic response was induced by soy isoflavones.19) Daidzein, in vitro, has been proven to increase the activation of murine lymphocytes.14) It has been found in mice that daidzein enhanced several immunological functions.15) The exact role isoflavones could play in terms of modulating immune activity therefore remains unclear, but extensive animal work showing their effects on immune parameters suggests that immune effects of genistein and daidzein are feasible. Although the mechanism by which genistein enhanced the expression of CD80 and CD86 has not been precisely established, our observation is of...
considerable interest since it could represent a new kind of immunomodulating activity of IFA.

The results in this study show that IFA could modulate the myeloid cell function. Effective immune responses are based on both the innate and adaptive immune systems. It has been reported that a number of food components are able to stimulate immune reactions mostly via the activation of innate immunity. It is interesting to note that isoflavone has regulatory activity toward the co-stimulatory function of antigen-presenting cells, suggesting a different role from those of other food components in enhancing immunity. Pathogen encounter by cells of the immune system represents a form of danger initially sensed by professional antigen-presenting cells that undergo specialization to prime naïve T and B lymphocytes, leading to a cellular or humoral response or both. There is substantial evidence that defined molecular events within antigen-presenting cells follow the biosynthesis of pro-inflammatory, inflammatory and anti-inflammatory cytokines/chemokines, notably the up-regulation of MHC Class I and II as well as co-stimulatory molecules (CD80 and CD86). These changes often promote the development of the potent effector T cell or antibody response needed to eradicate or contain pathogen-invaded tissue. These findings suggest that IFA could affect immune cells to contribute host defense against pathogens and to provide health benefits.

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References