Genistein Modulates Immune Responses and Increases Host Resistance to B16F10 Tumor in Adult Female B6C3F1 Mice


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ABSTRACT The isoflavone genistein (4,7,4'-trihydroxyisoflavone) is a phytoestrogen found in high levels in soy products that has been associated with decreased incidences of breast and prostate cancers. The potential effects of genistein on the immune system were evaluated in adult female B6C3F1 mice. Groups of mice were exposed to vehicle or genistein by gavage for 28 d. The doses of genistein used were 2, 6 and 20 mg/kg body. Consistent with the chemopreventive effect of genistein, exposure to this compound significantly increased host resistance to B16F10 tumor as reflected by a decrease in the number of lung tumor nodules after tumor cell injection at the middle and high dose levels. Inhibition of B16F10 tumor formation was not due to a direct effect of serum genistein and/or its metabolites on the proliferation of B16F10 tumor cells. When innate and acquired immune responses were evaluated, a dose-related increase of cytotoxic T-cell activity was observed in genistein-treated mice with significant changes observed at the middle and high dose levels. Furthermore, in vitro interleukin (IL)-2–stimulated natural killer (NK) cell activity was significantly enhanced in the high genistein dose group, although the basal NK cell activity was not affected. Although no effect on the mixed lymphocyte responses and anti-CD3 antibody-mediated splenocyte proliferation was observed, exposure to genistein significantly increased basal splenocyte proliferation. Exposure to genistein did not alter the activity of the mononuclear phagocyte system and the cytotoxic/cytostatic function of thioglycollate-recruited peritoneal cells on B16F10 tumor cells. Finally, exposure to genistein did not produce biologically meaningful changes in spleen immunoglobulin (Ig)M and IgG antibody-forming cell responses. In conclusion, genistein enhanced host resistance as evaluated in the B16F10 tumor model, which may be related to the increases in the activities of cytotoxic T cells and NK cells. J. Nutr. 131: 3251–3258, 2001.

KEY WORDS: genistein • cytotoxic T cell activity • natural killer cell activity • antibody forming cell responses • B16F10 tumor model

Ingestion of genistein (GEN)-containing soybean products in Asian populations has been associated with a reduction in the risk of breast and prostate cancers (1–4). The potential beneficial effects of GEN in the human population are supported by similar findings in experimental animals (5–7). Although GEN has been shown to inhibit the growth of a wide variety of tumor cells in culture by inhibiting the activity of some enzymes, e.g., protein tyrosine kinases and topoisomerase II, this effect of GEN has not been associated with antitumor effects in vivo (5,8,9). In contrast, there is a large amount of evidence to demonstrate that GEN has weak estrogenic effect on the reproductive systems, especially at low concentrations (10,11). However, it is currently unclear how phytoestrogen GEN exerts its chemopreventive effect.

One possible mechanism for GEN to inhibit tumor development may involve its effect on the immune system. In addition to estrogen-dependent tumors, GEN has been shown to be effective in preventing the development of tumors for which there is no strong evidence supporting a requirement for estrogen (12). Furthermore, in athymic mice, which lack the development of T cells, no inhibitory effect was observed on the growth of estrogen-dependent or estrogen-independent tumors when they were exposed to dietary GEN (13,14). Considering the importance of both innate immunity and acquired immunity in antitumor mechanisms, it was hypothesized that GEN could modulate immune responses in female B6C3F1 mice. To delineate the effect of GEN on differential immunologic responses in vivo, we have performed the following studies.
immune functions in vivo, GEN was administered to adult female B6C3F1 mice for 28 d by gavage, a relevant route of exposure. A series of assays related to the cellular or humoral components of the immune system were performed, utilizing the splenocytes from mice that had been exposed to GEN. The results indicated that GEN could increase host resistance to B16F10 tumor challenge, and this enhancement was associated with an increase in cytotoxic T-cell and natural killer (NK) cell activities.

MATERIALS AND METHODS

Animals and diets. Female B6C3F1 mice were obtained from Charles River Breeding Laboratories (Portage, MI) or Taconic Farm (Germantown, NY). Mice arrived at 4–6 wk of age and were quarantined upon arrival. The mice were housed four per cage in plastic shoe-box cages with hardwood chip bedding, and consumed Harlan Teklad Laboratory Diets (NIH 01; Madison, WI) and tap water from water bottles. All mice were housed in a controlled environment (25 °C and the relative humidity between 40 and 70%). The light:dark cycle was maintained on 12-h intervals. All animal procedures were conducted under an animal protocol approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IAUC).

Genistein (Sigma, St. Louis, MO) solutions were freshly prepared daily in 25 mmol/L Na2CO3 at concentrations of 0.2, 0.6 and 2 g/L (2). The mice were administered these GEN solutions or the vehicle (25 mmol/L Na2CO3) for 28 d by gavage (0.1 mL/10 g body), and this preparation of 51Cr-labeled P815 cells, as described (15). Briefly, the splenocytes at different dilutions were mixed with the target cells to obtain effector:target ratios of 200:1, 100:1, 50:1, 25:1, 12.5:1 and 6.25:1. The spontaneous release was determined by adding 100 μL of medium to 12 replicate cultures containing the targets. The maximum release was determined by adding 100 μL of the YAC-1 cells and 100 μL of 0.1% Triton X-100 to each of 12 replicate wells. NK cell-specific lysis (%) of 51Cr-labeled YAC-1 cells was used as the endpoint of the assay. For interleukin (IL)-2 augmented NK assay, single cell suspensions of individual spleens were prepared and cultured with 5 × 105 irradiated cells of L-cell (Chiron, Emeryville, CA) overnight, and then assayed for NK cell activity using 51Cr-labeled YAC-1 cells as the target cells.

The activity of the mononuclear phagocyte system. The functional activity of the mononuclear phagocyte system was measured as described (18). The mice were injected iv with 3H-sheep red blood cells (sRBC, 0.10 hematocrit in PBS at 100 μL/10 g body). Blood samples were taken every 3 min from the tail of each mouse; blood (5 μL) was dispensed into a tube containing 1 mL of water. The clearance of the labeled sRBC from the blood was determined over the first 30 min and the half-life was calculated on the basis of the amount of radioactive remaining in the blood at each time point. After 60 min, mice were killed, exsanguinated and different organs removed. The uptake of the labeled sRBC by various organs was determined by the amount of radioactive present. Radioactivity was measured using a γ-counter (LKB).

Recruited peritoneal macrophage activity. The activity of recruited peritoneal macrophages was determined as described previously by Geissler et al. (19) with slight modification. Briefly, 5 d before killing (during the genistein exposure period), the mice were injected intraperitoneally with 1 mL of a 10% thioglycollate solution. Mice were killed by a gentle cervical dislocation. The recruited peritoneal cells (PEC) were obtained by lavage using HBSS. Cells (1 × 103) from each mouse were dispensed to each well in 96-well plates and adhered for 2 h at 37°C in 5% CO2. The nonadherent cells were then rinsed off; the adherent cells (macrophages), unstimulated or stimulated with interferon-γ (IFN-γ; 1 × 104 U/L; PharMingen, San Diego, CA) plus lipopolysaccharide (LPS; 10 μg/L), were further cultured for 4 h. Plates were rinsed twice and incubated for 48 h with the addition of the B16F10 melanoma target cells (1 × 104/well). All wells were pulsed with 3H-thymidine 14–16 h before harvest. The procedures for harvesting were described above. Inhibition of B16F10 tumor cell proliferation was used as an indicator of recruited peritoneal macrophage activity.

Spleen immunoglobulin (Ig)M/IgG antibody response to the T-dependent antigen, sRBC. The primary IgM response to sRBC was assayed using the NK-sensitive target, Na51CrO4-labeled YAC-1 cells, as described with modification (17). Briefly, the splenocytes at different dilutions were mixed with the target cells to obtain effector:target ratios of 200:1, 100:1, 50:1, 25:1, 12.5:1 and 6.25:1. The spontaneous release was determined by adding 100 μL of medium to 12 replicate cultures containing the targets. The maximum release was determined by adding 100 μL of the YAC-1 cells and 100 μL of 0.1% Triton X-100 to each of 12 replicate wells. NK cell-specific lysis (%) of 51Cr-labeled YAC-1 cells was used as the endpoint of the assay. For interleukin (IL)-2 augmented NK assay, single cell suspensions of individual spleens were prepared and cultured with 5 × 105 irradiated L-cell (Chiron, Emeryville, CA) overnight, and then assayed for NK cell activity using 51Cr-labeled YAC-1 cells as the target cells.

Natural killer (NK) cell activity. The activity of NK cells was assayed using the NK-sensitive target, Na51CrO4-labeled YAC-1 cells, as described with modification (17). Briefly, the splenocytes at different dilutions were mixed with the target cells to obtain effector:target ratios of 200:1, 100:1, 50:1, 25:1, 12.5:1 and 6.25:1. The spontaneous release was determined by adding 100 μL of medium to 12 replicate cultures containing the targets. The maximum release was determined by adding 100 μL of the YAC-1 cells and 100 μL of 0.1% Triton X-100 to each of 12 replicate wells. NK cell-specific lysis (%) of 51Cr-labeled YAC-1 cells was used as the endpoint of the assay. For interleukin (IL)-2 augmented NK assay, single cell suspensions of individual spleens were prepared and cultured with 5 × 105 irradiated L-cell (Chiron, Emeryville, CA) overnight, and then assayed for NK cell activity using 51Cr-labeled YAC-1 cells as the target cells.

In vitro proliferation of B16F10 tumor cells. To determine whether GEN and/or its metabolites present in the serum of exposed mice had any effect on the proliferation of B16F10 melanoma cells, blood was collected from anesthetized mice by cardiac puncture. These mice had been exposed to the vehicle or GEN for 28 d as described. Serum was isolated and stored at −20°C until use. All procedures were performed in a sterile condition. For B16F10 cell proliferation assay, cells (1 × 105/100 μL) were cultured in the presence of 50% serum in complete RPMI 1640 medium supplemented with sodium bicarbonate, HEPES, t-glutamine, gentamicin and 2-mercaptoethanol. After 24 h of incubation at 37°C and 5% CO2, all wells were pulsed with 20 μL of 3H-thymidine (1:10 dilution). The plates were incubated for another 14–16 h before harvest. For harvesting, the supernatant was pipetted out of the wells and the cells in the wells were rinsed twice with Hank’s balanced salt solution (HBSS). Trypsin was added to all wells for 45 min to release the cells from the plates; all wells were then harvested using the Cambridge Cell Harvester (Cambridge, MA) with consistent mechanical scraping and agitation. The results were expressed as kBq/1 × 104 cells.

Cytotoxic T lymphocyte (CTL) activity. The assay for CTL activity was performed as described (16). The splenocytes from control and treated mice were washed once with HBSS, resuspended in Eagle’s minimal essential medium (E-MEM, Hazleton, Lenexa, KS). The cells were sensitized in vitro with mitomycin C-treated P815 mastocytoma cells at a responder:sensitizer ratio of 50:1 for 5 d at 37°C in 5% CO2. For the preparation of mitomycin C–treated P815 cells, cells were incubated in the dark with mitomycin C at a concentration of 50 μg/2 × 105 cells for 30 min at 37°C. Then the cells were washed four times. After the sensitization phase, cultured spleen cells were harvested and resuspended in E-MEM for determination of CTL activity. Na51Cr-labeled P815 cells (2 × 104 cells/100 μL) were cocultured in duplicate with 100 μL of graded numbers of splenic effector cells in U-bottom microtiter culture plates to yield a serial half-dilution of effector:target ratios from 25:1 to 0.75:1. For the preparation of Na51CrO4-labeled P815 cells, 15 × 105 cells were incubated with 18.4 MBq of Na51CrO4 for 60 min at 37°C with finger vortex every 15–20 min followed by four washes. After a 4-h incubation at 37°C and 5% CO2, the plates were centrifuged for 10 min at 250 × g. Supernatant from each well (100 μL) was removed and counted in a LKB gamma spectrophotometer (Gaithersburg, MD). Controls for spontaneous and maximum release were generated by culturing labeled target cells in the presence of either E-MEM medium or 0.1% Triton X-100, respectively.
and the GEN exposure groups. The percentages of lymphocytes and NK cells in the spleen were measured using flow cytometric analysis (22). For NK cells (NK1.1+CD3−), spleen single-cell suspensions were dual-labeled with fluorescein isothiocyanate (FITC) conjugated anti-mouse CD3 mAb (1:80; Becton Dickinson, San Jose, CA) and phycoerythrin conjugated anti-NK1.1 mAb (1:80; PharMingen) for 30 min on ice. The cells were washed and enumeration performed on a Becton Dickinson FACS can Flow Cytometer in which log fluorescence intensity was read gated on propidium iodide to eliminate dead cells and a forward scatter threshold high enough to eliminate RBC. The data were analyzed using the CellQuest software (Becton Dickinson). For B cells, the staining procedures were the same as those described for NK cells except that goat anti-mouse IgG (heavy and light chain specific) conjugated to FITC was used. Irrelevant, isotype matched antibodies were used as the control.

**Mixed leucocyte response to DBA2 spleen cells.** The assay was performed as described (23). One hundred microliters of splenocytes (1 × 10^7/L) were added to each well of a U-bottom microtiter plate (Costar 3799, Cambridge, MA). DBA/2 spleen cells were used as the allogeneic cells (stimulator) for the B6C3F1 mice (responder). Stimulator cells were treated with mitomycin C to render them unable to proliferate; the ratio of stimulators to responders has previously been optimized to be 4:1. The cells were cultured for 5 d, during the last 18 h in the presence of 36.8 kBq [3H]-thymidine. The cells were collected with a cell harvester and counted in a LKB liquid scintillation counter. The amount of [3H]-thymidine incorporated into the proliferating responder cells was expressed as kBq/10^5 cells.

**Anti-CD3 antibody-mediated spleen T-cell proliferation.** The proliferation of splenocytes in the presence of anti-CD3 antibody was performed as described above. A single spleen cell suspension was prepared and resuspended in RPMI medium supplemented with fetal bovine serum (10%), sodium bicarbonate, HEPES, l-glutamine, gentamicin and 2-mercaptoethanol. The splenocytes (5 × 10^6/well) were cultured in the microtiter wells coated with anti-CD3 antibody (1 mg/L; PharMingen) or in wells without antibody coating at 37°C at 5% CO2 and 95% humidity. Before harvest on d 3, the cells were pulsed with [3H]-thymidine for 18–24 h. The incorporation of [3H]-thymidine into the proliferating cells was expressed as kBq/5 × 10^5 cells.

**Statistical analysis.** The data were analyzed as follows. Bartlett’s test for homogeneity was used to select the type of analysis to be conducted. Homogeneous data were analyzed using a one-way ANOVA; when significant, Dunnett’s t test was used to determine differences between the experimental and the control groups. For nonhomogeneous data, a nonparametric ANOVA was used; when significant, differences between the control group and the experimental groups were determined using the Wilcoxon Rank Test. Jonckheere’s Test was used to test for dose-related trends across the vehicle and the GEN exposure groups.

### RESULTS

To determine the chemopreventive activity of GEN, the effect of this compound on host resistance in the B16F10 tumor model was evaluated. Adult female B6C3F1 mice were exposed to GEN at the dose levels of 2, 6 and 20 mg/kg, and the assay performed as described. The mean number of tumor nodules in the vehicle control mice was 198 (Fig. 1A). Exposure to GEN resulted in a dose-related decrease in pulmonary tumor formation. One possible explanation for the enhanced host resistance to B16F10 tumor was that GEN or its metabolites might directly inhibit the proliferation of B16F10 tumor cells. Thus, B16F10 tumor cell proliferation was evaluated in the presence of sera collected from vehicle- or GEN-exposed mice. The proliferation of B16F10 tumor cells did not differ among vehicle control and GEN treatment groups when the sera (50%) from treated mice were used to culture the B16F10 tumor. Mice were exposed to the vehicle (VH, 25 mmol/L Na2CO3) or GEN, and the host resistance to B16F10 tumor assayed as described. Values represent the mean ± SEM, n = 8. a, Significantly different from the control (VH); b, significantly different from the low dose group (2 mg/kg). (B) In vitro proliferation of B16F10 tumor cells. The proliferation of B16F10 tumor cells in the presence of serum (50%) obtained from mice (n = 8) that had been exposed to vehicle or genistein for 28 d.

CD8+ T cells play a central role in protection against tumor metastasis. Therefore, the CTL activity was examined using P815 mastocytoma cells as a target. Exposure to GEN increased CTL activity significantly in the middle and high dose groups (Fig. 2). When the data were expressed as specific activity, vehicle control mice had 45.9 lytic units (LU) in 10^7 spleen cells, and 1.2- and 1.6-fold increases were observed in the middle and high dose groups, respectively (Fig. 2A). When the data were expressed as total activity, vehicle controls had 625.8 LU in spleen, and 1.2- and 1.6-fold increases were observed in the middle and high dose groups, respectively (Fig. 2B). Na2CO3, the vehicle used in this study, did not affect the CTL activity compared with naïve controls. Exposure to GEN had no effect on the percentage of CD8+ T cells in the spleen (Table 1).

The CTL assay tests cell-mediated immunity by measuring the final differentiation of T cells to cytotoxic effector cells. This occurs after the proliferative response, e.g., mixed leucocyte reaction (MLR), takes place. To determine the mechanisms underlying the modulated T cell activity by GEN, the one-way MLR was evaluated using allogeneic lymphocytes, DBA/2 spleen cells. Exposure to GEN had no effect on the MLR as demonstrated by no differences in the proliferative response of the responders in the presence of stimulators.
NK cells play an important role in innate immunity against tumor growth; thus, the cytotoxicity of NK cells in GEN-exposed adult female B6C3F1 mice was determined. Exposure to GEN significantly increased in vitro IL-2-augmented NK cytotoxicity in the high dose group. Both the LU/10^7 cells (Fig. 3A) and LU/spleen (Fig. 3B) were significantly increased. However, GEN did not affect basal NK cell activity (data not shown). Furthermore, exposure to GEN had no effect on the percentage of NK cells in the spleen (Table 1).

Macrophages are another innate immune mechanism to control tumor growth. The functional activity of the mononuclear phagocyte system was measured by determining the vascular clearance of ^{31}Cr-sRBC and the uptake of the ^{31}Cr-sRBC by various organs including liver, spleen, lungs and thymus as described. The clearance/uptake of ^{31}Cr-sRBC as a measurement of the capacity of fixed macrophages has been widely used to evaluate the effect of various chemicals on the functional activity of the mononuclear phagocyte system (18). The kidneys are considered to be a nonphagocytic organ; therefore localization of ^{31}Cr-sRBC in the kidneys was determined for control purposes. In vehicle control mice, the half-life (T_{1/2}) of ^{31}Cr-sRBC was 10.9 min; exposure to GEN did not affect the vascular clearance of ^{31}Cr-sRBC. The percentage of uptakes of ^{31}Cr-sRBC by liver, spleen, lungs and kidneys were 19.7, 13.3, 1.4 and 2.1%, respectively. Exposure to GEN had no effect on the uptake of ^{31}Cr-sRBC by these organs when evaluated as either percentage of uptake or Bq/mg tissue (Table 3). For thymus, the treatment groups did not differ from the vehicle control separately, but a significant decreasing trend was observed when the data were expressed as percentage of uptake and Bq/mg (P ≤ 0.01 by Jonckheere's Test). Furthermore, exposure to GEN produced an increasing trend in the terminal body weight and the weights of liver, spleen and lungs (P ≤ 0.05 by Jonckheere's Test).

We also examined whether enhanced macrophage activity played a role in enhanced host resistance in the B16F10 tumor model by quantitating the cytotoxic/cytostatic activity of thioglycollate-recruited peritoneal macrophages, which is a direct extension of our observation of increased host resistance by GEN in this model. No significant effect was observed in the mice that had been exposed to GEN at dose levels of 2–20 mg/kg for 28 d regardless of whether the peritoneal macrophages were unstimulated or stimulated with IFN-γ and LPS (data not shown).

The IgM AFC response to the T-dependent antigen, sRBC, was also evaluated. Although GEN increased IgM AFC at the dose of 6 mg/kg (Table 4), no effect was observed in the high dose group. Furthermore, exposure to GEN had no effect on

![FIGURE 2](https://example.com/figure2.png)

**TABLE 1**

Effect of genistein on the percentage of differential splenocytes in female B6C3F1 mice^1,2^  

<table>
<thead>
<tr>
<th>Genistein (mg/kg)</th>
<th>Splenocytes</th>
<th>B cells (lg^+^)</th>
<th>CD4^+^CD8^-</th>
<th>CD4^-CD8^+</th>
<th>NK cells (NK1.1^+^CD3^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH</td>
<td>18.6 ± 0.7</td>
<td>53.5 ± 3.4</td>
<td>19.8 ± 0.6</td>
<td>12.0 ± 0.4</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>19.4 ± 0.8</td>
<td>58.3 ± 1.9</td>
<td>20.0 ± 0.6</td>
<td>12.0 ± 0.2</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>18.7 ± 0.6</td>
<td>52.2 ± 0.6</td>
<td>18.6 ± 0.9</td>
<td>11.1 ± 0.4</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>19.9 ± 1.1</td>
<td>56.9 ± 2.5</td>
<td>19.2 ± 0.8</td>
<td>11.4 ± 0.5</td>
<td>3.0 ± 0.1</td>
</tr>
</tbody>
</table>

^1^ Values represent the mean ± SEM, n = 8.  
^2^ The splenocytes were prepared from the mice that have been treated with vehicle (VH) or genistein for 28 d. NK, natural killer; Ig, immunoglobulin.

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that consumption of soy products that contain high levels of GEN (Table 1).

IgG AFC response (Table 4). The splenic B cell percentage was not altered by GEN (Table 1).

DISCUSSION

Both epidemiologic and experimental evidence suggests that consumption of soy products that contain high levels of GEN may have some beneficial health effects, including decreased incidences of breast and other hormone-associated cancers (1–4). To improve our understanding of GEN’s protective effect, we examined its effects on immune responses in adult female B6C3F1 mice. Consistent with the tumor inhibitory effect of GEN reported in humans, increased host resistance in the B16F10 tumor model was observed after exposure of mice to GEN by gavage, a relevant route of exposure, for 28 d. This is also in agreement with previous studies that oral administration of GEN inhibited B16F10 lung-tumor nodule formation (24) and decreased B16 tumor volume (6). For a 4-mo-old infant who consumes soy formula as directed by the manufacturers, ~6–9 mg/kg body of isoflavones can be achieved (25). A GEN concentration as high as 5 μmol/L in plasma can be reached in women who take tablets of soy extract (26). In our study, physiologically relevant doses of GEN (2–20 mg/kg) were employed. At the dose levels of 6 and 20 mg/kg, we demonstrated that GEN significantly decreased B16F10 lung tumor nodule formation.

There are several possible mechanisms that may be responsible for the inhibitory effect of GEN on B16F10 tumor formation. Some in vitro studies have demonstrated that GEN can directly inhibit the proliferation of tumor cells (6,27). However, this mechanism may not play an important role in our in vivo tumor model. As demonstrated in our study, GEN and/or its metabolites present in the sera of GEN-exposed mice had no effect on B16F10 proliferation. This suggested that either the serum levels of GEN and/or its metabolites in our experimental animals were not high enough to directly inhibit the proliferation of B16F10 tumor cells or they were in a form (e.g., protein-bound) that had no direct effect on the proliferation. Because GEN concentration in plasma is higher than that in lungs after oral dosing of [14C]GEN (28), the inhibitory effect of GEN on B16F10 lung tumor formation is unlikely due to a direct effect of GEN present in the lungs. It should be noted that GEN has been found to be an inhibitor of protein tyrosine kinases at high concentrations while acting as an estrogenic compound at low concentrations (4,29). Furthermore, there is evidence that inhibition of protein tyrosine phosphorylation prevented T cell–mediated cytotoxicity (30) instead of enhancing CTL activity as demonstrated in our study. Therefore, the enhancing effect of GEN on host resistance in the B16F10 tumor model is likely due to its function

![Figure 3](https://academic.oup.com/jn/article-abstract/131/12/3251/4686313)
as an estrogenic compound instead of an enzyme inhibitory agent.

In addition, the B16F10 tumor model requires the extravasation of melanoma cells from the cardiovascular system into the interstitium of the lungs by invading the subendothelial basement membrane (31). Genistein has been shown, in vitro, to inhibit the invasion of the extracellular matrix by mammary carcinoma cells (32), which may also play a role in our in vivo tumor model. However, this effect of GEN has been attributed to its inhibition of protein tyrosine kinases (31). As indicated above, the lack of effect on the proliferation of B16F10 tumor cells by GEN and/or its metabolites present in the sera of GEN-exposed mice suggests that enhanced host resistance to B16F10 tumor model was not due to these enzyme inhibitory effects.

Cytotoxic T cells, NK cells and macrophages are suggested to be the major immune mechanisms responsible for clearance and growth inhibition of B16F10 melanoma tumor (33–35). In this study, we demonstrated that GEN significantly increased the activity of CTL at the dose levels of 6 and 20 mg/kg. However, whether increased CTL activity is responsible for enhanced host resistance to tumors is currently unknown. Moreover, the mechanism underlying GEN-enhanced CTL activity remains to be defined. The expression of estrogen receptor protein or transcript in thymus and peripheral T cells has been demonstrated (36,37). When C3H/HeJ mice were treated orally with tamoxifen (an estrogen antagonist), enhanced CTL activity to H2712 carcinoma was observed (38). Genistein may function as an antiestrogen in a similar manner in our experimental model to enhance CTL activity. Although GEN had no effect on the MLR and anti-CD3 antibody-mediated splenocyte proliferation, an increased basal splenocyte proliferation was observed in both studies. What antigen and which type of cells contributes to this increase is currently unknown.

Lymphokine-activated killer cells have been shown to be successful in cancer immunotherapy (39). In our study, in vitro IL-2–augmented NK cell activity but not basal NK cell activ-

### TABLE 3

Effect of genistein on the mononuclear phagocyte system in adult female B6C3F1 mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>2</th>
<th>6</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life, min</td>
<td>10.9 ± 1.4</td>
<td>7.3 ± 0.4</td>
<td>11.6 ± 1.6</td>
<td>10.5 ± 1.1</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>25.2 ± 0.7</td>
<td>26.9 ± 1.3</td>
<td>27.8 ± 1.9</td>
<td>28.6 ± 1.3</td>
</tr>
<tr>
<td>Liver weight, mg</td>
<td>873 ± 36</td>
<td>955 ± 54</td>
<td>983 ± 54</td>
<td>1048 ± 57</td>
</tr>
<tr>
<td>% Uptake</td>
<td>19.7 ± 2.9</td>
<td>28.6 ± 2.6</td>
<td>24.6 ± 1.6</td>
<td>24.8 ± 2.1</td>
</tr>
<tr>
<td>Bq/mg</td>
<td>6.79 ± 0.94</td>
<td>9.67 ± 0.78</td>
<td>8.27 ± 0.43</td>
<td>8.03 ± 0.62</td>
</tr>
<tr>
<td>% Uptake</td>
<td>71 ± 4</td>
<td>86 ± 5</td>
<td>84 ± 5</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>Lung weight, mg</td>
<td>13.3 ± 2.1</td>
<td>12.3 ± 1.4</td>
<td>13.3 ± 2.2</td>
<td>12.7 ± 1.4</td>
</tr>
<tr>
<td>Thymus weight, mg</td>
<td>55.26 ± 6.55</td>
<td>47.58 ± 6.36</td>
<td>51.64 ± 7.33</td>
<td>51.36 ± 4.84</td>
</tr>
<tr>
<td>% Uptake</td>
<td>169 ± 7</td>
<td>203 ± 9</td>
<td>202 ± 19</td>
<td>188 ± 10</td>
</tr>
<tr>
<td>Bq/mg</td>
<td>2.54 ± 0.59</td>
<td>1.21 ± 0.20</td>
<td>1.91 ± 0.39</td>
<td>2.07 ± 0.59</td>
</tr>
<tr>
<td>Kidney weight, mg</td>
<td>63 ± 5</td>
<td>61 ± 4</td>
<td>70 ± 7</td>
<td>54 ± 5</td>
</tr>
<tr>
<td>% Uptake</td>
<td>0.050 ± 0.010</td>
<td>0.017 ± 0.002</td>
<td>0.031 ± 0.009</td>
<td>0.013 ± 0.003</td>
</tr>
<tr>
<td>Bq/mg</td>
<td>0.2 ± 0.24</td>
<td>0.09 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Bq/mg</td>
<td>308 ± 14</td>
<td>319 ± 12</td>
<td>315 ± 9</td>
<td>340 ± 14</td>
</tr>
<tr>
<td>% Uptake</td>
<td>21 ± 0.5</td>
<td>1.9 ± 0.1</td>
<td>2.4 ± 0.4</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Bq/mg</td>
<td>2.03 ± 0.27</td>
<td>1.91 ± 0.12</td>
<td>2.54 ± 0.47</td>
<td>1.91 ± 0.16</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8.
2 The half-life of 51Cr-sheep RBC (sRBC) in blood and the uptake of the 51Cr-sRBC into the liver, spleen, lungs, thymus and kidneys were determined by the amount of radioactivity in each organ as described.
3 There was a significant difference in dose-related trends among the vehicle and exposed groups.

### TABLE 4

Effect of genistein on antibody-forming cell (AFC) responses to sheep red blood cells in adult female B6C3F1 mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Genistein (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM AFC/10⁶ spleen cells</td>
</tr>
<tr>
<td>Genistein, mg/kg</td>
<td>VH</td>
</tr>
<tr>
<td></td>
<td>1180.6 ± 70.4</td>
</tr>
<tr>
<td></td>
<td>1148.5 ± 113.1</td>
</tr>
<tr>
<td></td>
<td>1914.3 ± 166.8a</td>
</tr>
<tr>
<td></td>
<td>1234.6 ± 126.5</td>
</tr>
<tr>
<td></td>
<td>2 ± 1a</td>
</tr>
</tbody>
</table>

1 The values represent the means ± SEM, n = 7 or 8. a Significantly different from all the other groups.
2 Mice were exposed to vehicle (VH) or genistein for 28 d. Four days before the assay, the positive control (PC) mice received cyclophosphamide [50 mg/(kg • d) four times (once per day)]. The number of immunoglobulin (IgM and IgG AFC in spleen was determined as described. Data are presented as the AFC/10⁶ spleen cells (specific activity) and as AFC × 10³/spleen (total spleen activity).
IMMUNOMODULATION BY GENISTEIN


