Effect of Dietary Soy Protein on Tumor Necrosis Factor Productivity in Macrophages from Nephritic and Hepatoma-Bearing Rats

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Summary The present study investigated the effect of dietary soy protein isolate (SPI) on tumor necrosis factor-α (TNF) productivity in peritoneal macrophages from nephritic and hepatoma-bearing rats. Dietary SPI significantly inhibited the elevated production of TNF by lipopolysaccharide-stimulated macrophages in nephritic and hepatoma-bearing rats compared with dietary casein, while it exerted no influence on the TNF productivity in normal rats. Removal of the minor components contained in SPI by ethanol extraction could significantly or partially restore the reduced TNF production caused by SPI in nephritic and hepatoma-bearing rats, respectively. These results suggest that dietary SPI could suppress the enhanced productivity of TNF associated with the progression of nephritis and hepatoma, and some factors existing in the ethanol extract of SPI are suggested to be involved in suppressing TNF productivity by macrophages.

Key Words ethanol-treated soy protein isolate, hepatoma, nephritis, soy protein isolate, tumor necrosis factor-α

Tumor necrosis factor-α (TNF) is the proinflammatory cytokine produced by activated macrophages and has several biological roles (1). Excess production of TNF is known to cause acute and chronic inflammation. Several reports suggest that TNF is related to inflammatory diseases such as glomerulonephritis (2, 3) and the promotion of tumors (4, 5). Thus, suppression of excess production of this cytokine is needed in inflammatory and tumor-bearing states. We have examined the effects of various dietary factors on hyperlipidemia or other symptoms observed in nephrotoxic serum-induced nephritic rats (6–8) and in AH109A hepatoma-transplanted rats (9–11). In these experiments, we have found that macrophages from AH109A-transplanted rats show marked enhancement of TNF productivity compared with those from normal rats, and that wheat gluten used as a protein source inhibits the productivity of TNF compared with milk casein in AH109A-transplanted rats (12).

Soy protein, another plant protein source, is reported to have beneficial effects on atherosclerosis progression and inflammation (13, 14). However, little is known about whether the production of proinflammatory cytokines such as TNF is altered by soy protein consumption in nephritic and tumor-bearing states. If altered, soy protein consumption is beneficial for the enhanced productivity of TNF accompanied with nephritis and tumor development. Thus, in the present study we investigated the effect of dietary soy protein on TNF productivity in nephritic and hepatoma-bearing rats. In addition, the effects of removal of isoflavones, saponins and other components from soy protein preparation by ethanol extraction were also assessed.

MATERIALS AND METHODS

Experimental design. This study was composed of six separate experiments. We first examined in experiment 1 whether TNF productivity by resident peritoneal macrophages was activated in nephritic rats as well as in hepatoma-bearing ones (12). In experiments 2, 3 and 4, we investigated the effects of dietary SPI and casein as protein sources on TNF productivity by macrophages in normal (experiment 2), nephritic (experiment 3) and hepatoma-bearing (experiment 4) rats. In experiments 5 and 6, we compared the effects of the SPI and ethanol-treated SPI diets on TNF productivity by macrophages in nephritic (experiment 5) and hepatoma-bearing (experiment 6) rats. All animal experiments were conducted in accordance with the guidelines established by the Animal Care and Use Committee of Tokyo Noko University.

Animals and diets. The nephritic rat model and hepatoma-bearing rat model used in our laboratory were established with Wistar rats (15) and Donryu rats (16), respectively. Thus, we used these rat strains in the present study. Male Wistar rats and male Donryu rats were obtained from Charles River Japan (Kanagawa, Japan) and NRC Haruna (Gunma, Japan), respectively. They were kept on a stock pellet and then a 20% casein
Table 1. Composition of experimental diets.

<table>
<thead>
<tr>
<th>Ingredient (g/100 g diet)</th>
<th>20C</th>
<th>20S</th>
<th>20SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Soy protein isolate (SPI)</td>
<td>—</td>
<td>20.0</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol-treated SPI</td>
<td>—</td>
<td>—</td>
<td>20.0</td>
</tr>
<tr>
<td>α-Cornstarch</td>
<td>51.3</td>
<td>51.3</td>
<td>51.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>17.0</td>
<td>17.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Mineral mixture (AIN-93G-MX)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture (AIN-93-VX)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

2. Fuji Oil Co., Osaka.
5. Hayashi Chemical Co., Tokyo.

diet (20C) ad libitum in an air-conditioned room with a temperature of 22 ± 2°C, a relative humidity of 60 ± 5%, and a light cycle from 08:00 to 20:00. The composition of basal and experimental diets was based on our previous reports (10, 12), and is summarized in Table 1. In the diets given to nephritic rats, sucrose was replaced with α-cornstarch as a carbohydrate source (6–8). Rats of 4 wk of age were used in all experiments. In experiment 1, male Wistar rats were divided into two groups of similar body weights (n=5). The rats of one group received a single intravenous injection into the tail vein of anti-rat kidney glomerular basement membrane (GBM) rabbit antiserum, which was produced by immunizing rabbits with the supernate of trypsin digested rat GBM (17, 18). The next day, the animals were subcutaneously immunized with rabbit γ-globulin (8 mg/rat. Sigma Chemical Co., St. Louis, MO, USA) in 0.2 mL Freund’s complete adjuvant (Wako Pure Chemical Industries, Ltd., Osaka, Japan) into the hind footpads as described previously (15, 19). Rats given neither antiserum injection nor rabbit γ-globulin immunization were regarded as the normal control. They were kept on the 20C diet until being killed on day 10. In experiment 2, male Wistar (experiment 2A) or Donryu (experiment 2B) rats were divided into two groups of similar body weights (n=6), and rats of each group were fed for 10 d (experiment 2A) or 14 d (experiment 2B) either the 20C or a 20% soy protein isolate (SPI) diet (Table 1). In experiment 3, Wistar rats with nephrotoxic serum-induced nephritis were divided into two groups (n=6) and were given either the 20C or the 20S diet for 10 days. In experiment 4, Donryu rats were divided into two groups (n=6) and all rats received subcutaneous implantation of 5 × 10^3 AH109A cells (obtained from Cell Resource Center for Biomedical Research, Tohoku University, Sendai, Japan) suspended in phosphate-buffered saline to produce a solid tumor on the back, as described previously (10, 12). Each group was given either the 20C or the 20S diet for 14 d. In experiments 5 and 6, we examined the effect of minor components-depleted SPI on TNF productivity by macrophages in nephritic and hepatoma-bearing rats. To remove isolavones and saponin from SPI, the SPI was treated with 70% ethanol, according to the methods of Kudou et al. (20), and insoluble materials were freeze-dried and kept at 4°C. From 100 g of soy protein isolate, 11.2 g of the ethanol-soluble materials was removed. The nephritic rats (experiment 5) or the hepatoma-bearing rats (experiment 6) were divided into three groups (n=5): the first group was given the 20C diet, the second group the 20S diet, and the third group a 20% ethanol-treated SPI (20SE) diet for 10 or 14 d, respectively. In experiments 1, 3 and 5, urine excreted by the rats was collected for 24 h from 09:00 of day 9 to day 10. All rats were deprived of their diets at 09:00 on the scheduled days but allowed free access to water until their sacrifice, which was conducted 4 h later by decapitation.

Isolation and culture of resident peritoneal macrophages. Resident peritoneal macrophages were harvested by lavage of the peritoneal cavity with RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan), containing 100 units/mL of penicillin G, 100 µg/mL of streptomycin and 2 mM L-glutamine (all from Wako Pure Chemical Industries) as described previously (12). The RPMI 1640 medium was prepared in endotoxin-free water (Hikari Pharmaceutical Co., Tokyo). The cells were washed twice with the medium at 4°C, and then resuspended in RPMI 1640 supplemented with 10% fetal calf serum (JRH Biosciences, Lenexa, KS, USA). Cells were seeded at 1 × 10^6 cells/well into 6-well plates (Nunclon, Roskilde, Denmark) and incubated at 37°C in 5% CO2/95% humidified air. After 2 h, the non-adherent cells were removed by washing three times with RPMI 1640, and the adhering cells were incubated for another 3 h in the presence of 1 µg/mL of lipopolysaccharide (LPS, from Salmonella typhimurium, obtained from Sigma Chemical Co.). LPS is known to be a potent stimulator for macrophages to secrete TNF. At the end of the incubation, the culture supernatant was harvested and stocked at −20°C until analysis. Cells were dissolved in 0.1% sodium dodecyl sulfate, and their DNA contents were measured fluorometrically as described previously (12). Cellular DNA contents were confirmed to be unchanged in all experiments (data not shown).

TNF assay. The TNF productivity (pg/µg DNA) in the culture supernatant was measured by an in vitro cytotoxic assay with L929 mouse fibroblasts as described previously (12). Data were represented as the relative values compared with those of the control group.

Statistical analysis. Data were expressed as the mean ± standard error (SE). Differences between two groups (in experiments 1–4) were analyzed by Student’s t-test. Multiple comparisons (in experiments 5 and 6) were performed by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple-comparisons test; p values less than 0.05 were considered significant.
RESULTS

In order to determine the effects of dietary proteins on TNF productivity in nephritic and hepatoma-bearing rats, we first examined in experiment 1 whether TNF productivity by resident peritoneal macrophages was enhanced in nephritic rats, because the LPS-induced production by macrophages has been found to be activated in hepatoma-bearing rats as already reported (12). Urinary protein excretion, the marker of the severity of nephritis, markedly increased in nephritic rats (305±33 mg/d/100 g body weight), while it was low in normal rats (4.0±0.6 mg/d/100 g body weight) at day 10. The LPS-induced production of TNF was significantly increased 10 d after the injection of anti-GBM antiserum as shown in Fig. 1. This result suggested that TNF productivity by resident peritoneal macrophages was enhanced in nephritic rats as well as hepatoma-bearing rats. From this result and our previous reports on measurement of transforming growth factor-β production in nephritic rats (7), we fixed the experimental period of 10 d in nephritic rats in the present study.

We next investigated in experiments 2, 3 and 4 the effects of dietary SPI and casein as a protein source on TNF productivity by macrophages in normal, nephritic and hepatoma-bearing rats. Food intake and body weight gain were unchanged between the 20C-fed and the 20S-fed rats in the normal state. In both diseased states, however, these were significantly lower in the 20S group than in the 20C group throughout this study (data not shown). Urinary protein excretion in nephritic rats (experiment 3) significantly decreased in the 20S group (341±14 mg/d/100 g body weight) compared to the 20C group (463±25 mg/d/100 g body weight). LPS-induced TNF production by macrophages is illustrated in Fig. 2. No change was seen in TNF production between the two dietary groups in the normal Wistar (Fig. 2A) and Donryu (Fig. 2B) rats. On the other hand, feeding of the 20S diet, as compared with that of the 20C diet, significantly reduced the production in nephritic rats (Fig. 2C) and hepatoma-bearing rats (Fig. 2D).

Finally, we prepared an ethanol-treated SPI, from which minor components such as soy isoflavones and saponin were depleted, and thereafter compared the effects of the SPI (20S) and ethanol-treated SPI (20SE) diets on LPS-induced TNF production by macrophages in nephritic and hepatoma-bearing rats. Food intake and body weight gain in the 20SE group were unchanged compared with the 20C group, but were also decreased in the 20S group as seen in experiments 3 and 4 (data not shown). Urinary protein excretion in experiment 5 was found to fall in the 20S group (296±34 mg/d/100 g body weight) compared to the 20C group (418±30 mg/d/100 g body weight), and this effect was maintained in the 20SE group (265±45 mg/d/100 g body weight). TNF production was confirmed to decrease in the 20S group compared with the 20C group in both experiments. Removal of the minor components from the 20S (20SE), however, significantly restored or tended to restore the LPS-induced production of TNF in nephritic (Fig. 3A) and hepatoma-bearing rats (Fig. 3B).

DISCUSSION

In the present study, we investigated the effects of dietary SPI and ethanol-treated SPI on TNF productivity in nephritic and hepatoma-bearing rats. Our results indicated that dietary SPI, compared with dietary
casein as a protein source, could inhibit the enhancement of TNF productivity by macrophages associated with nephritis and hepatoma implantation, whereas no significant change was seen between the two dietary groups in the productivity of the normal state. Similar results have been observed in wheat gluten, another plant protein source, which inhibits the productivity of TNF compared with casein in hepatoma-bearing rats, but not in normal ones (12), suggesting plant proteins, compared with animal protein like casein, could reduce the elevated production of TNF associated with inflammatory conditions. Various reports suggest that inflammatory responses could promote the development of glomerulonephritis (21, 22) and tumor (23, 24), and enhanced productivity of TNF is known to play a key role in acute and chronic inflammatory diseases; chronic TNF production is suggested to be an exacerbating factor for glomerulonephritis, because blockade of TNF prevents acute glomerular inflammation and progression of glomerulonephritis (25, 26). Likewise, proinflammatory cytokines such as TNF are related to the progression of tumors (4, 5). Thus, inhibition of excess TNF production is thought to have beneficial effects on inflammation and to inhibit the progression of glomerulonephritis and tumors. On the other hand, TNF has critical roles in host immune response to parasitic infections; because blockade of TNF during anti-TNF therapy is known to augment the risk of infections in patients with autoimmune diseases (27, 28), it is desirable that TNF productivity is not changed in normal conditions. Thus, dietary SPI is useful for reduction of TNF productivity because it inhibits only in inflammatory conditions but not in normal ones.

The SPI used in this study contains soy isoflavones as minor components and these are reported to mediate partially the beneficial effects of dietary SPI (29). Soy isoflavones such as genistein are reported to inhibit the production of TNF (30) and inflammatory responses (31). Soy isoflavones are also found to suppress nephritis-induced hyperlipidemia in our laboratory (32). From our results, removal of the minor components from SPI by ethanol extraction is suggested to restore or tend to restore the suppressed productivity of TNF by SPI in nephritic and hepatoma-bearing rats, respectively. These results demonstrate the inhibitory effect of dietary SPI on TNF productivity is mediated by the ethanol-extractable minor components. Recently, we have found that ethanol extract from SPI significantly suppressed the enhanced productivity of TNF in macrophages isolated from other inflammation model rats when added to the culture media (33). This report also supports our present results that the ethanol-extractable components in SPI mediate a reduction in the enhanced productivity of TNF by macrophages. Sakemi et al. (34) report that supplementation of semipurified ethanol-extractable components in soy protein (mainly consisting of isoflavones) to a casein-based standard diet has the ability to attenuate glomerular injury in Imai rats. Supplementation of ethanol-extractable materials in the SPI to the standard casein diet will explain more clearly their ability to inhibit the enhanced productivity of TNF. On the other hand, the SPI diet suppressed urinary protein excretion, a symptom of nephritis, compared with the casein diet. This effect of the SPI diet was maintained in the ethanol-treated SPI diet. These results suggest that improvement of proteinuria in nephritic rats is mediated by ethanol insoluble materials (mainly protein) rather than by ethanol-extractable materials. In this study, food intake was significantly reduced in the 20S group compared with the 20C group only in the diseased states. The possibility is not fully excluded that the reduction in food intake of the 20S group causes the inhibition of TNF productivity. A pair-feeding experiment will clarify the relation between food intake and TNF productivity.

In conclusion, dietary SPI suppresses the enhanced productivity of TNF in both nephritic and hepatoma-bearing rats but not in normal ones, and the ethanol-extractable components may contribute to the inhibition of TNF productivity in the SPI diet. The precise mechanisms and active component(s) for the inhibitory effect of dietary SPI on TNF productivity are currently unknown and remain to be elucidated.

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REFERENCES