FULL PAPER  Immunology

Enhancement of Antigen-Specific Immunoglobulin G Production in Mice by Co-Administration of L-Cystine and L-Theanine

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ABSTRACT. Supplementation with both cystine and glutamic acid increases the synthesis of glutathione (GSH), which has a marked effect on immune cell function, as compared with supplementation with either amino acid alone in human macrophages in vitro. As dietary glutamic acid is metabolized during intestinal transport, oral administration of L-theanine (γ-glutamylethylamide), which is metabolized to glutamic acid mainly in the liver, may act as a glutamic acid donor in vivo. The present study was performed to investigate the effects of oral administration of L-cystine and L-theanine on GSH levels and immune responses. Co-administration of L-cystine (200 mg/kg) and L-theanine (80 mg/kg) for 11 days before immunization significantly increased the levels of total GSH in the liver 6 hr after immunization compared with the levels in control mice. To examine the effects of administration of L-cystine and L-theanine on the balance of T helper (Th) 1/Th2 cell responses, the serum ratios of the Th1 cytokine, interferon (IFN)-γ and the Th2 cytokine, interleukin (IL)-10, were investigated. At 24 hr after immunization, co-administration significantly increased the IL-10/IFN-γ ratio compared with the ratios of the control and single-administration mice. Furthermore, co-administration before primary immunization significantly enhanced serum antigen-specific IgG levels. Taken together, these findings suggest that co-administration of L-cystine and L-theanine enhances antigen-specific IgG production partly through augmentation of GSH levels and Th2-mediated responses.

KEY WORDS: antigen-specific immune response, cystine, cytokine, glutathione, theanine.

Glutathione (GSH) is a tripeptide of cysteine, glutamic acid and glycine (γ-glutamyl-L-cysteinyl-glycine) and is a ubiquitous major antioxidant in vivo [14, 21, 26]. The antioxidative activity of GSH is exhibited by supplementing and removing reactive oxygen species generated in the living body, and many recent findings have indicated its importance in the immune system [8, 13, 26]. In the immune system, lymphocytes act as a defense mechanism against infection by bacteria and viruses. A reduction in the level of GSH in lymphocytes results in inhibition of their activity and proliferation. Thus, the level of GSH is correlated with lymphocyte activity and proliferation, and an appropriate intracellular GSH concentration of lymphocytes is believed to be important for normal functioning of the immune system [7, 8, 13]. In addition, it has been suggested that GSH enhances the functions of macrophages (Mφ), one class of antigen-presenting cells [5]. When an individual is infected, the cell-mediated immunity and humoral immune response are activated to initiate the defense mechanism, and the function of Mφ is to control the direction of this activation [6]. These findings also suggest that an increased GSH level is important in both cell-mediated immunity and humoral immune response, and this has also been demonstrated experimentally [10, 18].

Cysteine is a precursor of GSH, and its supply is rate-lim-
IFN-γ ratio in the serum as a reference. Finally, the impact of oral administration of L-cystine and/or L-theanine on antigen-specific antibody production was examined.

MATERIALS AND METHODS

Animals: Nine-week-old female BALB/c mice were purchased from Charles River Japan, Inc. (Yokohama, Japan). The animals were housed under specific pathogen-free conditions with food and water ad libitum and were kept on a 12:12 hr light:dark cycle. The mice were given a standard laboratory diet, CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan), which contained L-methionine (0.45 g/100 g diets) and L-cystine (0.35 g/100 g diets) as sulfur amino acids (data provided by Oriental Yeast Co., Ltd.). All animal experiments were performed using twelve-week-old mice. The experimental protocol was approved by the Ethical Commission for Animal Research of Ajinomoto Co., Inc.

Reagents: Dinitrophenylated dextran (DNP-dextran) was prepared by conjugation of dextran T-2000 (Amersham Biosciences, Piscataway, NJ, U.S.A.) to e-DNP-1-lysine hydrochloride (MP Biomedicals, Irvine, CA, U.S.A.) according to the standard method [12]. DNP-keyhole limpet hemocyanin (DNP-KLH) and incomplete Freund’s adjuvant were obtained from Merck (Darmstadt, Germany). Aluminum hydroxide was purchased from LSL (Tokyo, Japan). DNP-bovine serum albumin (DNP-BSA) was generously donated by Dr. Yasuhiro Takagi (Osaka Prefectural Institute of Public Health, Osaka, Japan). Rabbit antinouse IgM-horseradish peroxidase (HRP) and rabbit antinouse IgG (H+L)-HRP were obtained from Invitrogen (Carlsbad, CA, U.S.A.). Rat anti-mouse IgE (e chain specific)-HRP was obtained from Southern Biotech (Birmingham, AL, U.S.A.). Other chemicals were of commercially available reagent grade.

Administration of cystine and/or theanine: L-Cystine (Ajinomoto Co., Inc., Tokyo, Japan) was suspended in 0.5% carboxymethylcellulose sodium salt (CMC). L-Theanine (Taiyo Kagaku Co., Ltd., Yokkaichi, Japan) was dissolved in 0.5% CMC. The L-cystine and/or L-theanine suspension (cystine:theanine = 5:2; 10 mg/kg body weight) was orally administered to the mice every morning once a day for eleven days. Control mice were orally administered 0.5% CMC (10 ml/kg body weight) during the same period. On the day following the final oral administration, the body weights of the mice were measured, and then the mice were immunized. The weights of liver samples isolated from the mice were also measured.

Determination of plasma cystine and glutamic acid concentration: Plasma samples were collected from the mice at 0, 0.5, 1, 2, 4 and 6 hr after final administration. The plasma was mixed with a 2-fold volume of 5% trichloroacetic acid. Each sample was centrifuged and filtered to obtain the supernatant for amino acid analysis. The concentrations of cystine and glutamic acid in the plasma were determined using an automatic amino acid analyzer (L-8500; Hitachi Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions.

Immunization and evaluation of the DNP-specific IgM, IgG and IgE antibody: For analysis of IgM production, the mice received an intravenous inoculation of the antigen DNP-dextran (10 μg/mouse). For analysis of IgG production, the mice were intraperitoneally immunized with the antigen DNP-KLH (100 μg/mouse) in incomplete Freund’s adjuvant as the primary immunization. Four weeks after immunization, the mice were intraperitoneally immunized with DNP-KLH (50 μg/mouse) as secondary immunization. For analysis of IgE production, the mice were intraperitoneally immunized with the antigen DNP-KLH (4 μg/mouse) and aluminum hydroxide adjuvant (4 mg/mouse). All serum samples were collected using a standard method and were stored at −80°C until analysis. Evaluation of DNP-IgM and IgG antibody was conducted in accordance with a previous report [17]. The antibody titer was defined as the dilution rate calculated using the intercept and slope of the measured value for each dilution sample at the optimal optical density. The final titer of IgM or IgG in each sample was calculated as the mean value of two titers. Anti-DNP-IgE titers were determined by the following methods. Serum samples were diluted 1:100 with PBS. Protein A sepharose beads (Amersham Biosciences) were then added to the diluted serum samples. After the samples were mixed by a rotator at 4°C overnight, 100 μl of the supernatant was applied to an ELISA using rat anti-mouse IgE (ε chain specific)-HRP (×2,000 in PBS-T) according to the same protocols used for determination of DNP-IgM or DNP-IgG described above. The final antibody production of IgE in each sample was represented as the optical density.

Analysis of GSH: Liver samples were isolated from the mice at 6, 24 and 48 hr after immunization using DNP-KLH (4 μg/mouse) and aluminum hydroxide (0.2 mg/mouse). The isolated liver sample (0.1 mg) was homogenized with 1 ml of extraction buffer (5% sulfosalicylic acid and 5 mM EDTA). After centrifugation, the GSH and glutathione disulfide (GSSG) supernatants were collected and quantified using a Glutathione Assay Kit according to the manufacturer’s protocol (Cayman Chemical, Ann Arbor, MI, U.S.A.).

Analysis of serum IL-10/IFN-γ ratio: Serum samples were collected from mice at 0, 6, 24 and 48 hr after immunization using DNP-KLH (4 μg/mouse) and aluminum hydroxide (0.2 mg/mouse). The IL-10 and IFN-γ concentrations were analyzed using mouse IL-10 and IFN-γ antibody bead kits, respectively, according to the manufacturer’s instructions (Biosource International, Camarillo, CA, U.S.A.). The IL-10/IFN-γ ratio was calculated by the division of each cytokine concentration.

Statistical analysis: All statistical analysis was performed using the SigmaStat 3.1 software (Systat Software, Inc., Richmond, CA, U.S.A.). The data concerning the effect of the timing of co-administration on antigen-specific IgG antibody production were analyzed by two-way analysis of variance (ANOVA). Other data were analyzed by one-way ANOVA or one-way repeated measures ANOVA followed.
by the Tukey test as a multiple comparison test. A p-value below 0.05 was considered significant.

RESULTS

Influence of simultaneous oral administration of L-cystine and L-theanine on plasma cystine and glutamic acid levels: After oral administration of 280 mg/kg of L-cystine and L-theanine for 11 days, the plasma concentrations of cystine and glutamic acid were determined. The results indicated a 2.7-fold increase in the cystine concentration 0.5 hr after oral administration compared with that at 0 hr, and the concentration then decreased and gradually returned to the reference value after 4 hr (Fig. 1a). A 1.3-fold increase was observed in the plasma glutamic acid concentration 0.5 hr after oral administration, indicating that L-theanine functions as a supplier of glutamic acid and causes increases in the blood glutamic acid concentration in vivo (Fig. 1b). These results were observed after a single administration of L-cystine and L-theanine (data not shown).

Influence of oral administration of L-cystine and/or L-theanine on hepatic GSH level: L-Cystine and/or L-theanine were administered orally for 11 days, and the in vivo GSH level was then analyzed. The results shown in Table 1 indicated that the total GSH level increased in the L-cystine or L-theanine single-administration group 6 hr after antigenic stimulation compared with the control group, but the difference was not significant. However, in the L-cystine and L-theanine co-administration group, the total GSH level increased significantly compared with the control group. In addition, the GSH/GSSG ratio, which influences the Th1/Th2 ratio, was increased significantly in the co-administration and L-theanine single-administration groups 6 hr after antigenic stimulation compared with the controls. The increases in the total GSH level and GSH/GSSG ratio were greater in the co-administration group than in the single-administration group, but the difference was not significant. The hepatic GSH level was used as a reference to investigate the effects of co-administration of L-cystine and L-theanine for different ratios (data not shown); an effect was only seen at a ratio of L-cystine to L-theanine of 5:2.

Influence of oral administration of L-cystine and or L-theanine on serum IL-10/IFN-γ ratio: The effects of orally administered L-cystine and/or L-theanine on Th1/Th2 cell responses were investigated. As shown in Table 2, L-cystine/L-theanine co-administration and L-theanine administration significantly decreased the serum IL-10 levels compared with those controls before immunization. Furthermore, L-theanine administration significantly decreased the IL-10 levels 24 hr after immunization in comparison with those of the controls. At 6 hr or 48 hr after immunization, there was no significant difference in the serum IL-10 levels between control mice and either group of treated mice. However, there was no significant change in the serum levels of IFN-γ in either group of treated mice compared with the controls at any time point observed. In recent years, the importance of relative levels of IFN-γ and IL-10 in determining Th1/Th2 balance has been suggested [16, 23]; therefore, we analyzed the serum IL-10/IFN-γ ratio. The serum IL-10/IFN-γ ratio decreased significantly in the L-cystine/L-theanine co-administration group compared

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Fig. 1. Effect of co-administration of L-cystine and L-theanine on the plasma cystine and glutamate levels. Mice were orally administered 0.5% CMC as a control (open circle) or co-administered L-cystine (200 mg/kg) and L-theanine (80 mg/kg, filled circles) for 11 days. Plasma samples were collected from the mice at 0, 0.5, 1, 2, 4 and 6 hr after the final co-administration of L-cystine and L-theanine. In control mice, plasma samples were collected at 0 hr after the final administration. The plasma a) cystine and b) glutamate concentrations were analyzed using an amino acid analyzer. Each value represents the mean ± SE of five mice. The data were analyzed by one-way ANOVA followed by the Tukey test as a multiple comparison test. * Significant difference from mice 0 hr after the final co-administration (p<0.05).
Table 1. Effect of L-cystine and/or L-theanine administration on GSH in the liver

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-Cystine + L-Theanine</th>
<th>L-Cystine</th>
<th>L-Theanine</th>
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<tbody>
<tr>
<td>Total GSH [μmol/g tissue]</td>
<td>1.3 ± 0.1</td>
<td>2.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.4</td>
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<tr>
<td>6 hr</td>
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<tr>
<td>24 hr</td>
<td>2.0 ± 0.5</td>
<td>2.3 ± 0.2</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.3</td>
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<tr>
<td>48 hr</td>
<td>2.9 ± 0.7</td>
<td>3.4 ± 0.4</td>
<td>3.3 ± 0.5</td>
<td>2.8 ± 0.5</td>
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<tr>
<td>GSH/GSSG [ratio]</td>
<td>6.0 ± 0.7</td>
<td>15.5 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7 ± 0.7</td>
<td>14.2 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>6 hr</td>
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<tr>
<td>24 hr</td>
<td>6.9 ± 2.4</td>
<td>7.2 ± 0.9</td>
<td>7.7 ± 2.1</td>
<td>7.1 ± 1.1</td>
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<tr>
<td>48 hr</td>
<td>10.8 ± 3.0</td>
<td>14.6 ± 2.1</td>
<td>12.6 ± 1.8</td>
<td>10.7 ± 2.3</td>
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</table>

Mice were orally administered L-cystine (200 mg/kg) and/or L-theanine (80 mg/kg) for 11 days and then intraperitoneally immunized with DNP-KLH and aluminum hydroxide. Six, 24 and 48 hr after immunization, livers were isolated and homogenized. The concentrations of total GSH and the ratio of GSH/GSSG were analyzed in liver extracts using a glutathione assay kit. Each value represents the mean ± SE of four mice. The data were analyzed by one-way ANOVA followed by the Tukey test as a multiple comparison test. <sup>a</sup> Significant difference from control mice at each time point (p<0.05).

Table 2. Effect of L-cystine and/or L-theanine administration on serum IL-10/IFN-γ ratio

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-Cystine + L-Theanine</th>
<th>L-Cystine</th>
<th>L-Theanine</th>
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<tbody>
<tr>
<td>IL-10 [pg/ml]</td>
<td>30.9 ± 1.4</td>
<td>46.7 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.0 ± 5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.6 ± 2.5</td>
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<td>6 hr</td>
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<tr>
<td>24 hr</td>
<td>48.6 ± 7.4</td>
<td>52.2 ± 1.9</td>
<td>41.6 ± 2.4</td>
<td>40.2 ± 2.3</td>
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<tr>
<td>48 hr</td>
<td>37.1 ± 2.0</td>
<td>35.6 ± 1.9</td>
<td>43.5 ± 2.1</td>
<td>29.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IFN-γ [pg/ml]</td>
<td>4.1 ± 0.1</td>
<td>1.1 ± 0.8</td>
<td>8.8 ± 2.2</td>
<td>4.1 ± 0.1</td>
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<tr>
<td>6 hr</td>
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<tr>
<td>24 hr</td>
<td>6.1 ± 1.8</td>
<td>12.1 ± 7.0</td>
<td>5.0 ± 0.9</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>48 hr</td>
<td>4.1 ± 0.1</td>
<td>3.2 ± 0.6</td>
<td>5.1 ± 0.7</td>
<td>4.1 ± 0.1</td>
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<tr>
<td>IL-10/IFN-γ [ratio]</td>
<td>7.5 ± 0.3</td>
<td>6.6 ± 0.5</td>
<td>6.3 ± 0.6</td>
<td>7.7 ± 0.6</td>
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<tr>
<td>6 hr</td>
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<tr>
<td>24 hr</td>
<td>8.0 ± 0.2</td>
<td>4.3 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.8 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>48 hr</td>
<td>9.1 ± 0.5</td>
<td>11.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1 ± 0.1&lt;sup&gt;ad&lt;/sup&gt;</td>
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</table>

Mice were administered and immunized by the same protocol as shown in Table 1. Zero, 6, 24 and 48 hr after immunization, serum was collected, and the IL-10 and IFN-γ concentrations were investigated using a fluorescent microbead array system. The IL-10/IFN-γ ratio was calculated by dividing each concentration. Each value represents the mean ± SE of four mice. The data were analyzed by one-way ANOVA followed by the Tukey test as a multiple comparison test. <sup>b</sup> Significant difference from control mice at each time point (p<0.05, p<0.01, respectively). <sup>a</sup> Significant difference from control mice at each time point (p<0.01, p<0.001, respectively).

with the control and single-administration groups 6 hr after antigenic stimulation. However, 24 hr after antigenic stimulation the IL-10/IFN-γ ratio was increased significantly in the co-administration group compared with the control and single-administration groups. In addition, the IL-10/IFN-γ ratio was decreased significantly in the L-theanine single-administration group 24 hr after antigenic stimulation, but this was not seen in the L-cystine single-administration group. The IL-10/IFN-γ ratio 48 hr after antigenic stimulation was unaffected by oral administration of L-cystine and/or L-theanine.

Influence of oral administration of L-cystine and/or L-theanine on antigen-specific IgM antibody production: We investigated whether oral administration of L-cystine and/or L-theanine enhances antigen-specific IgM antibody production. In the co-administration group, IgM antibody production was increased compared with the control and single-administration groups, but the difference was not significant (data not shown). We also investigated the concentration dependency of the effects of L-cystine and L-theanine on IgM antibody production. L-Cystine and L-theanine were administered orally at doses of 140, 280 and 560 mg/kg for 11 days. IgM antibody production exhibited the greatest increase 4 days after antigenic stimulation with co-administration at 280 mg/kg (139.1 ± 40.5% vs. controls), although the difference was not significant (data not shown).

Influence of simultaneous oral administration of L-cystine and L-theanine on antigen-specific IgG antibody production: We examined whether co-administration of L-cystine and L-theanine confers a general preventive effect against infection and the influence on IgG antibody production with strong antigen specificity. L-Cystine and L-theanine were administered orally at doses of 140, 280 and 560 mg/kg for 11 days. As shown in Fig. 2, no significant increase was observed 7 days after secondary antigen stimulation with administration of 140 or 560 mg/kg, but a significant
increase in IgG antibody production was observed at a dose of 280 mg/kg. The results 14 days after antigenic stimulation indicated a 1.2-fold increase compared with 7 days after stimulation in the control group. No significant increase was observed in the co-administration group compared with the controls. We then administered a dose of 280 mg/kg L-cystine and L-theanine orally for 11 days before and after primary antigen stimulation to investigate the influence of timing of L-cystine and L-theanine administration on IgG antibody production. As shown in Fig. 3, a significant increase in IgG antibody production was observed when oral administration occurred before primary antigen stimulation (F=4.613, P=0.039). On the other hand, IgG antibody production was unaffected when oral administration occurred after primary antigen stimulation (F=0.451, P=0.506).

Influence of simultaneous oral administration of L-cystine

<table>
<thead>
<tr>
<th>Table 3. Effect of co-administration of L-cystine and L-theanine on antigen-specific IgE antibody production</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>---------</td>
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<tr>
<td>Anti-DNP-IgE antibody production [O.D.]</td>
</tr>
<tr>
<td>Day 7 0.74 ± 0.14*</td>
</tr>
<tr>
<td>Day 14 1.14 ± 0.27*</td>
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<tr>
<td>Day 21 1.27 ± 0.27*</td>
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</table>

Mice were orally administered L-cystine (200 mg/kg) and L-theanine (80 mg/kg) for 11 days and then intraperitoneally immunized with DNP-KLH and aluminum hydroxide. Serum was collected on the indicated days after immunization, and antigen-specific IgE antibody production was analyzed by ELISA. Each value represents the mean ± SE of 6 mice. The data were analyzed by one-way repeated measures ANOVA followed by the Tukey test as a multiple comparison test. *Significant difference from the IgE levels on day 0 in each group (p<0.05).
and L-theanine on antigen-specific IgE antibody production: Finally, we examined IgE antibody production, which is believed to be closely associated with allergic reaction in vivo. As shown in Table 3, significant increases in IgE antibody production were observed in the control group after 7, 14 and 21 days compared with the reference value measured after 0 days of antigenic stimulation. In the co-administration group, a significant increase was only observed 21 days after antigenic stimulation. In the co-administration group, decreases in IgE antibody production were observed in all blood samples taken at different times, although the decrease was not significant.

DISCUSSION

The results of the present study clearly indicated that co-administration of L-cystine and L-theanine significantly enhances the GSH level in the liver and significantly increases the serum IL-10/IFN-γ ratio. In addition, co-administration was shown to significantly enhance antigen-specific IgG antibody production.

As shown in Table 1, co-administration significantly increased the GSH level in the liver. However, this effect was limited to the early stage (6 hr) after antigenic stimulation. In addition, the increases in the total GSH level and GSH/GSSG ratio in the liver induced by co-administration tended to be higher than those seen in the single-administration groups. There were no differences between groups in GSH level or GSH/GSSG ratio before antigenic stimulation (data not shown), and the total GSH level and GSH/GSSG ratio of the control group decreased from 4.2 ± 0.2 to 1.3 ± 0.1 μmol/g tissue and from 25.9 ± 1.2 to 6.0 ± 0.7, respectively, after antigenic stimulation. These observations agree with those of a previous report that LPS treatment decreases the GSH level in the liver [3], and the effect of co-administration is believed to occur only when the GSH level is decreased after antigenic stimulation, as observed with LPS treatment. As shown in Fig. 1, there was an approximately 2.7-fold increase in the plasma cystine level after co-administration. The plasma level of glutamic acid increased by approximately 1.3-fold after co-administration. These results suggest that an abundance of substrates, such as cysteine and glutamic acid, is necessary for recovery from a reduced GSH level resulting from antigenic stimulation in mice compared with the control group or the single-administration group, and this aids in early recovery (6 hr after antigenic stimulation) from a reduced GSH level resulting from antigenic stimulation. The above discussion is supported by the results of a previous study indicating a 37% increase in intracellular GSH level when glutamic acid was added during cystine treatment in an experimental system using human Mφ in vitro [24].

Many previous studies have indicated that the intracellular GSH level modifies cytokine production in monocytes and macrophages [11, 22]. As shown in Table 2, co-administration resulted in a Th1-dominant pattern compared with the control and single-administration groups, and this was found to become a Th2-dominant pattern by 24 hr. Recently, it was reported that S-adenosylmethionine, a precursor of GSH, increases the intracellular GSH concentration. In LPS-stimulated RAW cells from a monocyte cell line, S-adenosylmethionine inhibited TNF-α production but enhanced IL-10 and IL-6 production [27]. In the same report, it was suggested that N-acetylcysteine (NAC), which supplies the cysteine necessary for GSH synthesis, decreases the production of these 3 cytokines. These findings indicate that increases in the intracellular GSH level alone cannot explain the modification of cytokine production. In addition, GSH is known to increase both cell-mediated immunity and the humoral immune response. This suggests that GSH induces either Th1 or Th2 depending on the condition of the cell in vivo. Thus, from the above findings, we concluded that detailed analysis of the mechanism of promotion of GSH synthesis, Th cytokine production modification and increases in antibody production induced by oral administration of L-cystine and/or L-theanine will lead to clarification of the relationship between cytokine production and the GSH level.

Activated native T cells first express both Th1 and Th2 cytokines; however, inappropriate cytokine expression ceases immediately, and differentiation toward either Th1 or Th2 is determined. This has been reported to occur within 24 to 48 hr of activation of the Th cell [20]. Based on this finding, we believe that differentiation toward Th2 was promoted by 24 hr after antigenic stimulation in the present study. In the L-theanine single-administration group, Th1 was dominant 24 hr after antigenic stimulation. As ethylamine, a metabolite of L-theanine, induces secretion of Th1 cytokines from γδ T cells [4, 15], it is possible that this enhancement is due to cell-mediated immunity in the L-theanine single-administration group. This also exhibits the different effects of co-administration and single administration of L-theanine.

Co-administration induces a significant increase in antigen-specific IgG antibody production. Ross et al. reported that adenylyl cyclase toxin derived from Bordetella pertussis and LPS synergistically promote IL-10 production from innate immunocytes and induce Th2 and T regulatory 1 (Tr1) cells to enhance IgG1 antibody production [25]. As shown in Table 2, our findings also indicate that the IL-10/IFN-γ ratio increases significantly 24 hr after antigenic stimulation by co-administration and that a Th2 response is induced. On the other hand, it has been demonstrated that γδ T cells may become efficient antigen-presenting cells in an in vitro experimental system, and they are believed to function as an interface between the innate and acquired immune systems. Ethylamine generated by hydrolysis of L-theanine has been reported to activate these γδ T cells [2, 4]. The findings described above indicate that co-administration may promote activation of the innate immune system after antigenic stimulation and may enhance antigen-specific IgG antibody production. In the present study, we also found that co-administration before, but not after, primary antigen stimulation resulted in a significant increase in IgG...
antibody production. This result also indicates that γδ T cells, which can act as antigen-presenting cells, may have an important role in enhancing the IgG antibody production induced by co-administration before primary antigen stimulation. In addition, according to our data, co-administration enhanced IgG antibody production without influencing the IgE level. IL-10 secreted from Tr1 cells has been reported to be a key regulator causing the shift from IgE antibody production to IgG4 production and to correct inappropriate immune response [29]. As our findings indicate a significant increase in the IL-10/IFN-γ ratio 24 hr after antigenic stimulation, our results showing that co-administration enhances IgG antibody production without influencing the IgE level are not in conflict with the findings mentioned above.

The intracellular GSH concentration is lower in the lymphocytes of older animals, and when GSH is supplied from an external source, cell proliferation is induced more efficiently in the splenocytes of older animal compared with those of younger animals [9]. It has been suggested that antibody production responses against foreign antigens are lower in older individuals [19]. These findings suggest that it is meaningful to analyze the effects of administration of l-cystine and l-theanine or the mechanism of action in older animals. Animal diseases related to the increased longevity of pets and infections in domestic animals have become an issue in recent years. l-Cystine and l-theanine supplementation may increase the GSH level, which decreases with aging or infection, and may enhance antibody production.

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REFERENCES


