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Journal of Nutritional Biochemistry 23 (2012) 526–531

Journal of Nutritional Biochemistry

Dietary supplementation with high dose of epigallocatechin-3-gallate promotes inflammatory response in mice☆

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Received 3 October 2010; received in revised form 24 January 2011; accepted 7 February 2011

Abstract

Epigallocatechin-3-gallate (EGCG) from green tea has been indicated to have anti-inflammatory activity. However, most of the evidence is in vitro studies in which EGCG is often added at levels unachievable by oral intake. With few exceptions, in vivo studies along this line have been conducted in animal models of diseases, and the results are inconclusive. In this study, we fed C57BL/6 mice a diet containing 0%, 0.15%, 0.3% or 1% (w/w) EGCG for 6 weeks. Contrary to the assumption that EGCG would reduce inflammatory response, mice fed 0.15% and 0.3% EGCG diet exhibited no change while those fed 1% EGCG diet produced more proinflammatory cytokines tumor necrosis factor-α, interleukin (IL)-6, and IL-1β and lipid inflammatory mediator prostaglandin E_2 in their splenocytes and macrophages (MΦ) and less IL-4 in splenocytes. Spleens from the mice fed 1% EGCG diet also had higher proportions of regulatory T cells, MΦ, natural killer (NK) cells and NKT cells compared to those from mice fed the other diets. These results suggest that high intake of EGCG may induce a proinflammatory response, and this change may be associated with a disturbed homeostasis of immune cells involving changes in both function and number of specific immune cell populations. While the mechanisms and clinical significance for this effect of EGCG remain to be investigated further, these data suggest the need for defining accurate EGCG dose limits to induce an anti-inflammatory effect since current data indicate that higher doses would produce an inflammatory response.

Keywords: Green tea; EGCG; Inflammation; Splenocytes; Macrophages

1. Introduction

Green tea consumption has been suggested to have health benefits in preventing or mitigating the development of a variety of diseases including cardiovascular [1], autoimmune [2–5], inflammatory [6,7], and neurodegenerative diseases [8,9] as well as obesity [10–13], and cancer [14–19] . These effects of green tea are mainly attributed to its most abundant and biologically active catechin, (−)-epigallocatechin-3-gallate (EGCG). Inflammation has been shown to play an important role in the initiation and/or development of these diseases, and thus viewed as a target for preventive and therapeutic strategies. Therefore, it has been hypothesized that the purported beneficial effect of EGCG may be associated with its anti-inflammatory property. Studies in both in vitro and in vivo settings have been conducted to address this issue. However, the efficacy of green tea/EGCG, particularly with regard to effective dose(s), and the reports on the correlation between disease manifestation and the levels of inflammatory markers/mediators have been inconsistent. A majority of the studies were conducted in various in vitro models, for the most part using immortalized tumor cell lines. For example, EGCG was shown to inhibit the production of proinflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in human monocyte cell line U937 cells [20], and TNF-α in human monocyte cell line THP-1 cells [21], murine macrophage (MΦ) cell line, J774.1 cells [22] and RAW264.7 cells [23]. Another important proinflammatory mediator, prostaglandin (PG) E_2, and the key rate limiting enzyme for its synthesis, cyclooxygenase (COX)-2, were also inhibited by EGCG in human chondrocytes [24] and various tumor cell lines [25–27]. The anti-inflammatory effect of EGCG has been linked to its suppressive effect on the activation of NF-κB, a

Abbreviations: APC, allophycocyanin; Con A, concanavalin A; EGCG, epigallocatechin-3-gallate; FITC, fluorescein isothiocyanate; IFN, interferon; IL, interleukin; MΦ, macrophage; LPS, lipopolysaccharide; PE, phycoerythrin; PG, prostaglandin; Th, T helper; TNF, tumor necrosis factor.

☆ Funding sources: Supported by the US Department of Agriculture, National Institute of Food and Agriculture grant 2010-65200-20360, and the US Department of Agriculture, Agriculture Research Service contract 58-1950-7-707. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the US Department of Agriculture.

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doi:10.1016/j.jnutbio.2011.02.006

master transcription factor controlling activation of the genes for most inflammatory molecules [3,28–31].

As is often the case for many other phytochemicals, EGCG has limited bioavailability after ingestion. Results from human studies have shown that oral administration with 525 mg EGCG increased plasma level of EGCG to 4.4 μmol/L [32], and a single oral administration of EGCG at varied doses ranging from 50 to 1600 mg resulted in maximum plasma concentrations at 0.28 to 7.4 μmol/L after 1.3 to 2.2 h [33]. In an animal study, after mice were intragastrically given a single dose of EGCG (50–2000 mg/kg BW), the plasma levels of EGCG were dose-dependently increased up to 4.17μg/ml (9μmol/L) [34]. Based on these results, EGCG levels within 10 μmol/L range should be considered physiologically relevant. However, the majority of in vitro studies have used EGCG at much higher concentrations, typically more than 50μmol/L. Consequently, extrapolation of these results in order to define what EGCG would do in vivo has been questioned. Animal studies using disease models and a limited number of human trials have evaluated the in vivo effect of EGCG supplementation. For example, in mice, oral administration or peritoneal injection of EGCG was shown to attenuate symptoms and pathologies of experimental arthritis [4,5], colitis [6,7], experimental autoimmune encephalomyelitis (EAE, an animal model for human multiple sclerosis) [3] and murine model for human Sjogren's syndrome (an autoimmune disease) [35]. However, information about the effect of EGCG on plasma levels or ex vivo production of inflammatory markers (cytokines, COX-2 and transcription factors such as NF-κB) reported in those studies is incomplete and often inconsistent. Many factors contribute to the inconsistent and inconclusive results, including the health status of subjects studied, the form and dose of supplements, the supplementation route and duration, and ex vivo stimulation conditions.

Currently, green tea products are consumed by both healthy people and those suffering from different diseases. The impact of EGCG may depend on the health status of the subjects as well as the dose used. Studies thus far have mostly focused on hosts suffering from diseases. No information is available regarding the impact of EGCG on healthy subjects, the majority of green tea product consumers. Furthermore, most of those studies have utilized one dose level in their green tea product supplements. Since hepatotoxicity, which results from consumption of green tea products, has been reported (see review by Mazzanti et al. [36]), a complete dose-response study cannot be conducted in humans. Thus, we undertook the present study in which healthy adult mice were fed diets containing none or one of three doses of EGCG for 6 weeks while their general health condition, inflammatory responses and change in cellular profile of peripheral lymphoid tissue (spleen) were examined. We report here that while EGCG consumption at moderate levels does not alter the inflammatory response in a healthy host, high dose of EGCG significantly elevates production of several proinflammatory markers. This effect of EGCG is accompanied by significant weight loss without visible toxicity as assessed by the histological examination of several key organs.

2. Materials and methods

2.1. Animals and feeding regimen

Specific pathogen-free male C57BL/6JNIA mice (6–9 months) were purchased from the National Institute on Aging colonies at Harlan Sprague Dawley Inc. (Indianapolis, IN). Mice were individually housed in cages maintained at a constant temperature and humidity with a 12-h light/dark cycle. Mice were randomly divided in four groups (n=16/group) and pair-fed the AIN 93 diet supplemented with 0%, 0.15%, 0.3% or 1% EGCG (w/w) for 6 weeks. EGCG (TEAVIGO containing >95% EGCG) was kindly provided by DSM Nutritional Products (Kaiseraugst, Switzerland). In order to pair feed, mice were initially given a weighed portion of food daily. If any mouse did not eat the entire portion of food, the weighed portion for all mice was decreased to the amount that this mouse ate the previous day. If all the mice consumed all of the diet, then the quantity of diet given was increased until an individual mouse did not consume all the food. By group feeding, we decreased the variability among the mice both within each diet group and among the diet groups. All mice were observed daily for clinical signs of disease, and body weight was recorded weekly. At the end of study, mice were killed by CO_2 asphyxiation and exsanguination. Mice exhibiting tumors, splenomegaly, or skin lesions were excluded from the study. All conditions and handling of the animals were approved by the Animal Care and Use Committee of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University and conducted according to the NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Splenocyte and MΦ isolation

After mice were euthanized by CO_2 asphyxiation, spleens were aseptically removed. Single cell suspensions were prepared by gently disrupting spleens between two sterile frosted glass slides. Splenocytes were isolated via centrifugation (300×g), and red blood cells were lysed using Gey's reagent. Cells were cultured in RPMI 1640 (BioWhittaker, Walkerville, MD) medium, supplemented with 5% heat-inactivated fetal bovine serum, 25 mmol/L HEPES, 2 mmol/L glutamine, 100 KU/L penicillin and 100 mg/L streptomycin (all from Gibco Invitrogen, Grand Island, NY). Peritoneal exudate cells were obtained by peritoneal lavage and enriched for MΦ using the method of Kumagai et al. [37]. Peritoneal MΦ prepared in this manner was at least 90% pure, as assessed by the expression of surface marker F4/80.

2.3. Cellular composition of splenocytes

The percentages of major constituent cell types in spleens were determined using fluorescent-activated cell sorting (FACS) analysis. Splenocytes (1×10^6 cells/sample) were stained with the following antimouse antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (T cells), phycoerythrin (PE)-conjugated anti-CD19 (B cells), PE-conjugated anti-CD4, FITC-conjugated anti-CD8 (T suppressor or cytotoxic cells), allophycocyanin (APC)-conjugated anti-CD25 (IL-2 receptor α chain), FITC-conjugated anti-natural killer-1.1 (NK-1.1 cells), APC-conjugated anti-TCRαβ, PE-conjugated anti-CD11c and APC-conjugated antimouse F4/80 (MΦ). F4/80 antibody was from Caltag (Burlingame, CA) and all the other antibodies were from BD Pharmingen (San Diego, CA). Stained cells were analyzed on a FACSCalibur (BD Biosciences), and the results were analyzed using the Summit software, version 4.0 (DakoCytomation, Fort Collins, CO).

2.4. Cytokine and PGE_2 production

Splenocytes (4×10^6 cells/well) in 24-well culture plates (Becton Dickinson Labware) were cultured in the presence of concanavalin A (Con A, 1.5 mg/L) or lipopolysaccharide (LPS, 1 mg/L, Sigma) for 24 h for IL-6, TNF-α, and PGE_2 production, or in the presence of Con A (1.5 mg/L) or immobilized anti-CD3 (5 mg/L) in combination with soluble CD28 (2 mg/L) (referred to as anti-CD3/CD28) for 48 h for IL-2, IFN-γ, IL-4 and IL-10 production. MΦ (~5×10^5 cells/well) were incubated in 24-well plates in the presence of LPS (1 mg/L) for 24 h.

Cell-free supernatants were collected at the end of incubation and stored at −70°C for later analysis. All cytokines were measured using ELISA. The reagents for IL-2, IFN-γ, IL-10, IL-4, IL-6 and TNF-α assays were from BD Pharmingen, and those for IL-1β assay were from R&D system (Minneapolis, MN). Cells were lysed by repeated freeze/thaw cycles. Total cell proteins were analyzed using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Secreted and cell-associated IL-1β was analyzed in supernatants and cell lysates, respectively. PGE_2 was measured using radioimmunoassay as previously described [38]. All the concentrations of cytokine and PGE_2 by MΦ were normalized with total cell protein.

2.5. Statistical analysis

All results were expressed as means ± S.E.M. Statistical analysis was conducted using Systat 10 statistical software. Significant differences were determined using ANOVA followed by Tukey's HSD post hoc procedure. Significance was set at P<.05.

3. Results

3.1. General condition of mice

Mice in all diet groups remained healthy throughout the experiment. Daily food intakes of group pair-fed mice varied from 3 to 3.6 g/day. At the start of the study, body weight did not differ among the diet groups. However, supplementation with 0.15%, 0.3% or 1% of EGCG for 6 weeks dose-dependently prevented weight gain or caused weight loss (1% EGCG). This effect of EGCG became significant after 1 week in the 1% EGCG group, 2 weeks in the 0.3% EGCG group and 4 weeks in the 0.15% EGCG group (Fig. 1). Otherwise, mice in all diet groups appeared

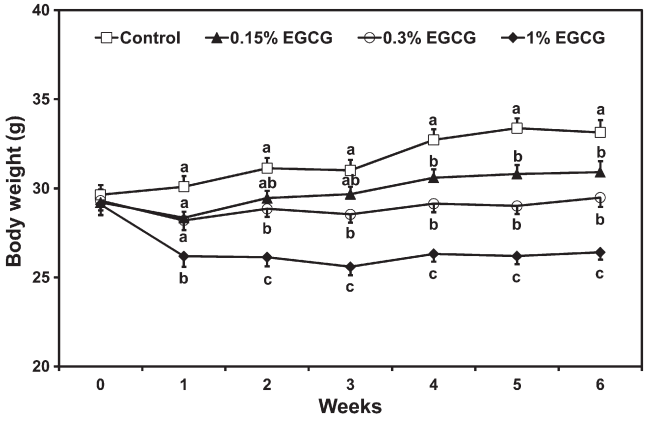

Fig. 1. Effect of EGCG supplementation on body weight of mice. Data are presented as means ± S.E.M., n=16/group. The values bearing different letters at the same time point significantly differ at least at P<.05 by ANOVA with Tukey's HSD post hoc test.

to be equally healthy and active. In addition, histological examination of heart, kidney and liver revealed no sign of toxicity in any group (data not shown).

3.2. Immune cell populations in spleen

Different immune cells have their unique functions, and one of their most significant functions is to produce cytokines and other soluble mediators, which determine or regulate the nature and magnitude of immune response. By itself, the phenotype information of peripheral lymphoid tissue is an important parameter in assessing the immunologic effect of an intervention, but it also serves to help explain a potential change in cytokine profiles should they occur after the intervention. While no significant difference in spleen immune cell population was observed in mice fed 0.15 and 0.3% EGCG, spleens from mice fed 1% EGCG diet had significantly lower total B cells ($CD19^+$) and higher regulatory T cells (Treg, $CD25^+/CD4^+$), MΦ ($F4/80^+$), NK1.1 ($NK\ 1.1^+$) and NKT ($NK\ 1.1^+/TCR\alpha\beta^+$) cells compared to those from mice fed the control diet (Fig. 2). Percentages of total, $CD4^+$ and $CD8^+$ T cells in spleen were not affected by EGCG supplementation of any dose.

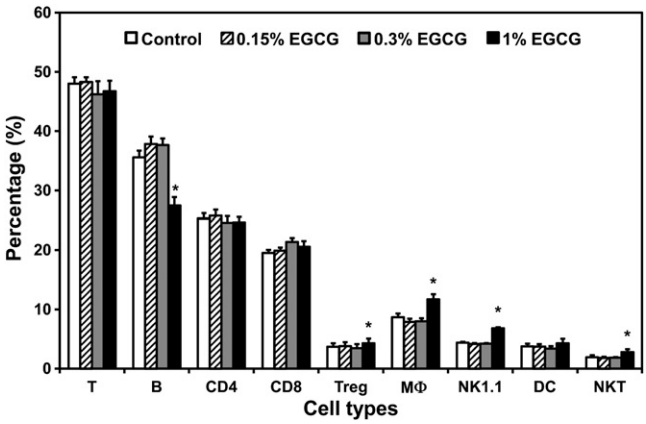

Fig. 2. Effect of EGCG supplementation on immune cell populations in spleen. Splenocytes (1×10^5/samples) were stained with fluorescence-conjugated mAbs against surface molecules, and percentages of each population were determined by flow cytometry as described in "Materials and Methods." Values are means ± S.E.M., n=16/group. *Significantly different from the control at least at P<.05 determined by ANOVA with Tukey's HSD post hoc test.

3.3. Production of inflammatory mediators by splenocytes

T cells produce a variety of cytokines. Based on the type of cytokines they predominantly produce, T cells are classified into their functional subsets. IFN-γ and IL-4 are hallmark cytokines for Th1 and Th2, respectively; IL-10 is widely viewed as Th2 as well as a Treg-associated cytokine. Th1 response is a major proinflammatory component, whereas Treg and Th2 are anti-inflammatory by nature. T cell cytokines can regulate activation of cells in innate immunity, such as MΦ and NK cells, which are sources of conventional proinflammatory mediators. Thus, T cell cytokine profiles could provide useful information to help assess the inflammatory state. In this study, compared to the control group, mice in the 1% EGCG group and not in the other EGCG groups had higher Con A- or anti-CD3/CD28-stimulated splenocyte production of Th1 cytokine IFN-γ and lower anti-CD3/CD28-stimulated production of Th2 cytokine IL-4 (Table 1). Compared to the control group, EGCG supplementation did not affect IL-2 (Th1 cytokine) and IL-10 production. LPS- or Con A-stimulated secretions of IL-6, TNF-α and PGE_2 were significantly higher in the 1% EGCG group than in any other group.

3.4. Production of inflammatory mediators by MΦ

MΦ are the most abundant source of major inflammatory cytokines and lipid mediators; therefore, their ability to produce these molecules is widely used as a hallmark by investigators in numerous in vitro and ex vivo studies to assess the inflammatory state. Since MΦ are a major constituent cell type in total splenocytes, it is likely that the observed effect of EGCG on splenocytes' cytokine and PGE_2 production may actually reflect a direct effect of EGCG on MΦ. To confirm this, we isolated MΦ from mice fed different doses of EGCG and determined the production of these molecules. Similar to the results observed in splenocytes, peritoneal MΦ from mice fed 1% EGCG diet produced significantly more IL-6, IL-1β and PGE_2 than those of mice in any other group. IL-10 and TNF-α production was comparable among all groups (Table 2).

4. Discussion

Studies have suggested that green tea EGCG has an anti-inflammatory activity, which may underlie its alleged benefit in

Table 1
Effect of EGCG supplementation on cytokine and PGE_2 production by splenocytes

Outcomes	Diet			
	Control	0.15% EGCG	0.3% EGCG	1% EGCG
IL-2 (pg/ml)				
Con A	192 ± 22^{ab}	144 ± 15^{a}	194±20^{ab}	225±30^{b}
Anti-CD3/CD28	166±35^{ab}	190±28^{ab}	231±55^{a}	87±10^{b}
IFN-γ (ng/ml)				
Con A	8.9±2.6^{a}	8.1±2.7^{a}	13.9±3.5^{ab}	20.6±5.3^{b}
Anti-CD3/CD28	222±25^{a}	196±27^{a}	256±39^{a}	357±57^{b}
IL-4 (pg/ml)				
Con A	4.5±1.3	6±2.7	7.7±3.1	10.9±4.5
Anti-CD3/CD28	187±21^{a}	182±21^{a}	164±23^{ab}	114±10^{b}
IL-10 (pg/ml)				
Anti-CD3/CD28	4037±511	3681±507	3455±423	3066±470
IL-6 (pg/ml)				
Con A	682±70^{a}	631±69^{a}	669±67^{a}	1313±159^{b}
LPS	491±42^{a}	440±39^{a}	428±35^{a}	865±83^{b}
TNF-α (pg/ml)				
Con A	88±14^{a}	83±11^{a}	134±19^{a}	245±53^{b}
LPS	256±37^{a}	231±17^{a}	250±21^{a}	541±68^{b}
PGE_2 (pg/ml)				
Con A	670±154^{a}	443±74^{a}	527±87^{a}	7725±3361^{b}
LPS	1194±192^{a}	1019±94^{a}	998±160^{a}	5213±1053^{b}

Values are means±S.E.M., n=16/group. The values bearing different superscript letters^{a,b} in the same row significantly differ at least at P<.05 by ANOVA with Tukey's HSD post hoc test.

Table 2
Effect of EGCG supplementation on cytokine and PGE_2 production by peritoneal MΦ

Outcomes	Diet			
	Control	0.15% EGCG	0.3% EGCG	1% EGCG
	(pg/μg protein)			
IL-6				
Medium	0.9 ± 0.2	1.3 ± 0.5	1.1 ± 0.3	1.8 ± 0.3
LPS	87 ± 13[a]	93 ± 10[a]	92 ± 10[a]	138 ± 14[b]
TNF-α				
Medium	ND	ND	ND	ND
LPS	5.6 ± 0.8	5.5 ± 0.4	4.9 ± 0.4	6 ± 0.5
IL-1β				
Medium	ND	ND	ND	ND
LPS				
Secreted	2.1 ± 0.3[a]	2.9 ± 0.3[ab]	2.6 ± 0.3[a]	3.9 ± 0.5[b]
Cell-associated	9.5 ± 1.5[a]	10.3 ± 1.0[a]	12.6 ± 1.2[ab]	15.7 ± 2.2[b]
Total	11.6 ± 1.7[a]	13.2 ± 1.2[a]	15.2 ± 1.4[ab]	19.6 ± 2.3[b]
PGE2				
Medium	0.8 ± 0.2[ab]	0.5 ± 0.1[a]	0.5 ± 0.1[a]	1.3 ± 0.3[b]
LPS	115 ± 13[a]	138 ± 13[a]	177 ± 19[a]	289 ± 29[b]
IL-10				
Medium	ND	ND	ND	ND
LPS	2.6 ± 0.5	2.6 ± 0.6	3 ± 0.7	3.4 ± 0.5

Values are means ± S.E.M., n=16/group. The values bearing different superscript letters[a,b] in the same row significantly differ at least at $P<.05$ by ANOVA with Tukey's HSD post hoc test. ND: not detectable.

inflammatory diseases and diseases in which inflammation is implicated for their pathogenesis. In this study, we sought to define whether dietary EGCG supplementation affects inflammatory response in a dose-dependent pattern. Unexpectedly, we found that EGCG at the two lower doses (0.15%, 0.3%, w/w in diet) did not significantly affect the capacity of two major sources of inflammatory molecules, splenocytes and MΦ, to produce the pro- or anti-inflammatory cytokines tested and PGE_2; however, its administration at a high dose (1%) in fact significantly elevated production of TNF-α, IL-6, IL-1β, IFN-γ and PGE_2. Since all of these molecules are known to be inflammatory mediators, these results suggest that consumption of EGCG at high doses such as used in this study may promote, rather than attenuate, the inflammatory response in healthy adult mice.

Given the sharp contrast of our findings to the general belief that EGCG is anti-inflammatory, the interpretation and implication of these results need to be addressed carefully. The studies determining the anti-inflammatory effect of in vivo green tea extract/EGCG administration are almost exclusively conducted in animal models of diseases. In this report, we will only discuss the studies in which EGCG was administered orally in order to provide a direct comparison of our study results since other methods of EGCG administration can result in varied bioavailability. In collagen-induced arthritis, mice fed a 0.2% green tea polyphenolic fraction in drinking water had reduced levels of TNF-α, IFN-γ and COX-2 expression in their arthritic joints [4]. EGCG gavage (50 mg/kg daily) attenuated elevation in serum TNF-α and IFN-γ levels and colonic NF-κB expression induced by acetic acid in colitis mice [7]. EGCG gavage (300 μg/mouse twice daily) given to EAE mice reduced the ex vivo TNF-α production of lymph node cells stimulated by PMA and ionomycin [3]. In these studies, the symptoms and pathologies of the disease were improved by EGCG supplementation. In general, the doses of EGCG used in these studies are lower than our medium dose (0.3% in diet). Why the anti-inflammatory effect of EGCG was observed in these studies but not in ours is unclear. One possibility is that the induced inflammation in these disease models elevated the basal levels of inflammatory status, which may have rendered the animals more responsive to EGCG treatment.

We also observed that 1% EGCG actually increased the production of inflammatory cytokines and PGE_2. This increased inflammatory response, which was induced by a high-dose EGCG, may help explain the unexpected results observed by Weisburger et al. [39], where contrary to their hypothesis, they observed increased tumorogenesis by EGCG. In that study, the authors found that in an azoxymethane-induced tumor model, rats fed a high dose of green tea extract (3600 ppm) for 43 weeks had significantly higher tumor multiplicity, while those on a low dose (360 ppm) showed no difference compared to the control animals. Another study used a carcinogen-induced tumor model in rats [40]. The results showed that in the rats fed a diet containing 0.1% or 1% green tea catechins (78.8% total catechins and 58.4% EGCG) for 33 weeks after tumor initiation, green tea catechins enhanced rather than inhibited colon carcinogenesis while not affecting lung and thyroid carcinogenesis. A more recent study [41] using a mouse model of chemical-induced colitis and colon carcinogenesis showed that compared to mice fed the control diet, those fed high doses (0.5 and 1%) of green tea polyphenols (GTP) had more severe colitis as well as higher mucosal levels of inflammation markers IL-1β and macrophage-migrating inhibitory factor (MIF). By contrast, mice fed lower doses (0.1 and 0.25%) of GTP had lower mucosal levels of IL-1β and MIF, but no difference in colitis was observed. It has been suggested that inflammation might be a risk factor in the initiation and development of certain tumors including colon cancer [42,43], though a causal relationship requires further investigation. Thus, the dose-specific modification of proinflammatory products by EGCG as observed in the current study might explain the varied impact on colon cancer reported in those studies using different doses of EGCG.

In the present study, we used spleen cells, a mixture of cell populations containing all types of mature immune cells, and peritoneal MΦ, a relatively pure population of MΦ after enrichment. The ability of each cell type to produce cytokines and PGE_2 varies owing to their different regulatory mechanism. Therefore, by comparing the change in cellular composition, we could estimate whether the increased production of proinflammatory cytokines and PGE_2 is due to an increased number of the cells that produce them, an increased capacity of cells to synthesize them, or both. IL-2 is exclusively produced by T cells; IL-4 is mainly produced by T cells but is also produced by mast cells. EGCG supplementation did not affect the percentage of total T cells, $CD4^+$ T cells, or $CD8^+$ T cells in spleen, but it inhibited IL-4 production by spleen cells. This suggests that the effect of EGCG on IL-4 production is probably mediated by affecting the function rather than the number of T cells. IFN-γ, a hallmark Th1 cytokine, is produced by both T cells and NK cells. In this study, EGCG did not alter the proportion of T cells in spleen, but significantly increased those for NK and NKT cells; the magnitude of observed increase in IFN-γ production is comparable to that in NK and NKT populations. Together, these data suggest that EGCG-induced increase in IFN-γ production is mainly due to the presence of more NK and NKT cells. The mechanisms for EGCG-induced increase in NK and NKT cells specifically are yet to be determined.

Proinflammatory cytokines TNF-α, IL-6 and anti-inflammatory cytokine IL-10 are mainly produced by MΦ and also, to a lesser degree, by T cells. EGCG supplementation enhanced production of TNF-α and IL-6 but did not affect IL-10. Since the MΦ proportion was increased about 30% by EGCG, which is smaller than the increase in TNF-α and IL-6 (2- to 3-fold), these data suggest that the higher level of these cytokines in mice fed EGCG compared to the control is due to both increase in number of MΦ and their ability to synthesize these cytokines. This is also supported by the observation that EGCG increased PGE_2 production in splenocytes by 5- to 10-fold since MΦ are the main producer of PGE_2 in spleen (little or none from T cells).

In peritoneal MΦ, consistent with the findings in splenocytes, EGCG increased production of proinflammatory cytokines (IL-1β and IL-6) and PGE_2, while having no effect on anti-inflammatory cytokine IL-10. However, TNF-α was not affected by EGCG, and the EGCG-induced increase in PGE_2 production was less dramatic than the one

observed in splenocytes. This differential response probably reflects the complex interaction among different cells in spleen. It is known that one important function of IFN-γ is to activate MΦ, which in turn manifest a greatly up-regulated activity including a burst of synthesis of inflammatory mediators [44–48]. Thus, it can be speculated that upon stimulation, increased IFN-γ production in the EGCG-treated group, probably due to higher percentage of NK and NKT cells as discussed above, provides a more robust stimulatory signal to MΦ. This, combined with an already increased percentage of MΦ in the spleen of EGCG-treated group, would result in a more dramatic increase in production of inflammatory mediators by splenocytes compared to purified peritoneal MΦ.

The observed proinflammatory effect of EGCG in this study seems to be paradoxical to what EGCG has been implicated in the case of obesity. It is well documented that obesity induces a low-grade inflammation, which is believed to play a key role in the development of metabolic syndrome including diabetes [49]. Antiobese and antidiabetic effect of EGCG has been proposed, mainly based on animal studies, and this proposed health benefit has become one of the reasons that EGCG is most favored as a supplement by the public. In this study, we used pair-feeding so that food intake was essentially the same for mice in all the groups. However, EGCG consumption resulted in weight loss in a dose-dependent manner. This observation agrees very well with the previous studies showing that dietary EGCG at 1% in C57BL/6 mice for 5 months [13], and at 0.5% and 1% in NZB mice for 4 weeks [11] prevented high fat diet-induced gain in body weight and fat mass. In both studies, food intake was the same between mice fed high fat and high fat plus EGCG. The inhibitory effect of EGCG on body weight and fat mass in high fat-induced mice has also been convincingly reported by other investigators [10,12]. The mechanisms appear to involve decreased energy/lipid absorption and lipogenesis, and increased fat oxidation [10–13]. In the above-mentioned studies, EGCG has been shown to improve glucose tolerance [10,13,50]. It is not known whether the effect of EGCG on body weight/fat mass and glucose tolerance is associated with its effect on inflammation status since inflammation markers were not measured in those studies except for a reduced plasma MCP-1 observed in EGCG-fed mice [10].

In order to determine whether or not our results have potential application to human health, the human equivalent of the doses used in this study needs to be determined. Based on the average mice's consumption of 3 g/days, diets containing the medium dose (0.3%), or 3 g EGCG /kg diet, provided mice with a daily intake of 9 mg EGCG, or 300 mg/(kg BW/d) for a mouse of 30 g (average BW in the current study). When this dose is converted from mice consuming 12 kJ/day to humans consuming 2000 kJ/day by using isocaloric calculation [34,51], it is roughly equivalent to 22 mg/(kg BW/day) in humans, or 1540 mg EGCG per day consumed by a 70-kg person. Likewise, the low dose (0.15%) and high dose (1%) of EGCG for mice in this study are equivalent to human daily consumption of 770 and 5040 mg, respectively. A cup of tea contains 150−180 mg EGCG; commercially available EGCG supplements contain up to 350 mg EGCG/tablet. Thus, the low dose (0.15%) and medium dose (0.3%) used in mice in the current study are achievable in humans by consuming high doses of supplements or by drinking large quantities (5–10 cups or 1 to 2 L/day) of tea. While EGCG at these doses did not affect inflammatory response in mice, it did reduce weight gain. Our results suggest that these doses may represent a safe range for human consumption of EGCG to gain health benefits such as weight control without introducing a risk of elevated inflammation. Although EGCG consumption by humans in an amount equivalent to the high dose (1%) used in mice is less likely to be ingested in daily diet, it is achievable by taking mega doses of EGCG supplements. Therefore, until human studies have proven otherwise, it is prudent to caution against consumption of high doses of EGCG because of its possible adverse effect.

Studies on green tea have used varied forms of supplements: green tea (brewed tea or tea powder), green tea extracts, tea polyphenols or purified EGCG. It would be interesting to know whether we would have observed the same effects of EGCG had it been administered in the form of tea in which EGCG coexists with many other constitutive components. Since this information is presently unavailable, we can only speculate that perhaps EGCG's effectiveness might vary due to the presence of other bioactive components as well as the assumed interaction among them. It has been suggested that EGCG is more bioavailable when administered together with other green tea catechins, and the combined administration has a synergistic inhibitory effect on cancer cell growth [52]. Furthermore, the method of taking in green tea/EGCG, e.g., via diet or a bolus formulation, is also a factor to consider when comparing the results from different studies.

In summary, in this study, we report the effect of dietary supplementation with different doses of EGCG on inflammatory response. Contrary to common belief, we found that a high dose (1%) of EGCG has a proinflammatory effect as demonstrated by increased production of several proinflammatory cytokines as well as the lipid inflammatory mediator PGE_2. Thus, one should bear it in mind that dose is critical in determining the direction and magnitude of EGCG's effect on inflammatory response. While the underlying mechanism and clinical relevance of these findings are yet to be determined, these results suggest that caution should be used in recommending a high dose of EGCG for consumption.

Acknowledgments

The authors would like to thank Stephanie Marco for her assistance in the preparation of the manuscript.

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