Epigallocatechin-3-Gallate Directly Suppresses T Cell Proliferation through Impaired IL-2 Utilization and Cell Cycle Progression 1–3

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Abstract

Previously, we demonstrated that in vitro epigallocatechin-3-gallate (EGCG) supplementation inhibited T cell response in mouse spleen cells. In this study, we confirmed this effect of EGCG in mice fed 0.3% EGCG for 6 wk. A coculture with all the combinations of preincubating antigen-presenting cells and T cells with or without EGCG showed that EGCG suppressed antigen-induced T cell proliferation, mainly through a direct effect on T cells. To determine the mechanisms for this effect of EGCG, we stimulated purified mouse T cells with anti-CD3/CD28 in the presence of EGCG (2.5–15 μmol/L) and found that EGCG dose-dependently inhibited cell division and cell cycle progression and this effect of EGCG was more pronounced in CD4+ than in CD8+ T cells. Interleukin (IL)-2 concentrations in EGCG-treated cell cultures showed no difference up to 24 h but were higher in the cultures at 48 h compared with the untreated control cells. However, intracellular staining showed no difference between EGCG-treated and untreated control cells in IL-2 synthesis, but EGCG-treated cells expressed less IL-2 receptor (IL-2R) compared with untreated control cells. EGCG did not affect mRNA expression of IL-2 and IL-2R. These results indicate that EGCG-induced IL-2 accumulation in 48 h cultures is due to its reduced utilization. In summary, EGCG directly inhibits T cell proliferative response to both polyclonal and antigen-specific stimulation. CD4+ cells are more responsive to EGCG than CD8+ cells. Future studies should determine the effect of EGCG on CD4+ cell subsets to assess its application in T cell-mediated autoimmune diseases. J. Nutr. 140: 1509–1515, 2010.

Introduction

Green tea and its bioactive components, catechins, in particular epigallocatechin-3-gallate (EGCG), have a variety of health-promoting and disease-preventing benefits. The effect of tea on immune function, however, has not been well studied. Of the limited number of studies reported, a great majority of them used in vitro supplementation with tea extracts or catechins. For example, in vitro supplementation with EGCG was shown to inhibit proliferation of spleen B and T cells (1). Likewise, in vitro supplementation with green tea extract inhibited proliferation of murine lymphocytes (2) and production of neopterin, a marker for cellular immunity, by human peripheral blood mononuclear cells (3). Studies have also shown that in vitro supplementation with EGCG suppressed maturation of mouse bone marrow-derived (4) and human monocytes-derived (5) dendritic cells (DC), the most important antigen-presenting cells. The allogeneic T cells cocultured with EGCG-treated DC also exhibited decreased proliferation (4,5). Taken together, these results suggest that EGCG could have a suppressive effect on cell-mediated immune response. However, most, if not all, of these studies have used EGCG at high concentrations (typically >50 μmol/L), which are much higher than the reported peak plasma levels achieved after oral consumption of green tea/EGCG by humans (6,7) or mice (8). In a recent study (9), we reported that EGCG at physiologically relevant concentrations (2.5–10 μmol/L) dose-dependently inhibited splenocyte proliferation stimulated by concanavalin A (Con A), a T cell mitogen. We also observed that T cell division and cell cycle progression were inhibited by EGCG (9). However, as in other previously reported studies, T cell response was induced and measured in total splenocytes, a mixture of multiple cell types. In such a mixture of cells, accessory cells (mainly macrophages and DC) play a role in facilitating T cell activation and consequent proliferation.

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4 D.W. and S.N.M. share senior authorship.
5 Abbreviations used: APC, allophycocyanin; Con A, concanavalin A; DC, dendritic cell; EGCG, epigallocatechin-3-gallate; IL, interleukin; IL-2R, interleukin-2 receptor; OVA, ovalbumin; PE, phycoerythrin; TCR, T cell receptor
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Because EGCG has been shown to affect the function of accessory cells present in total splenocytes, we could not determine whether the effect of EGCG was due to its direct impact on T cells or due to an indirect effect through modifying the function of accessory cells or both. Furthermore, if EGCG is proved to have a direct effect on T cells, the underlying cellular and molecular mechanisms mediating its T cell-suppressive activities are not well understood.

Although the concentrations of EGCG used in our previous study are physiologically relevant, i.e. those achievable through oral intake, it remains to be ascertained if similar effects would be observed following oral administration of EGCG. Thus far, the effect of in vivo EGCG supplementation on T cell-associated functions have been conducted in specific disease animal models (10–15), but the impact of in vivo EGCG supplementation on T cell responses of a healthy host has not been demonstrated. Therefore, in this study, we investigated: 1) the efficacy of in vivo supplementation with EGCG in healthy mice; 2) the direct effect of EGCG on T cell function using purified T cells; and 3) the underlying mechanisms of EGCG-induced effects on T cell function.

Materials and Methods

Mice. Specific pathogen-free C57BL/6 mice (6–9 mo) were purchased from Harlan Sprague Dawley, and DO11.10 and BALB/c mice (4–6 mo) were purchased from Jackson Laboratory. Mice were individually housed in cages maintained at a constant temperature and humidity with a 12-h-light-dark cycle. Water and nutritionally adequate mouse nonpurified diet (Teklad 7012, Harlan Teklad) were provided ad libitum. In the feeding study, mice were fed the experimental diets as described below. All mice were observed daily for general health and clinical signs of disease. At the end of the study, mice were killed by CO₂ 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.

Dietary EGCG supplementation. Mice were randomly divided into 3 groups (n = 9/group) and pair-fed the AIN 93M diet (16) supplemented with 0, 0.15, or 0.3% EGCG (wt/wt) for 6 wk. EGCG (TEAVIGO containing 95% EGCG) was kindly provided by DSM Nutritional Products. To conduct the pair feeding, mice were initially given an isocaloric diet as described below. All mice were observed daily for general health and clinical signs of disease. At the end of the study, mice were killed by CO₂ 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.

In vitro EGCG supplementation. EGCG (Sigma) was dissolved in RPMI 1640 medium (Biowhittaker). Purified T cells were preincubated with EGCG (0, 2.5, 5, 10, or 15 μmol/L) in a 37°C and 5% CO₂ incubator for 2 h before stimulation.

Splenocyte isolation and T cell purification. After mice were killed by CO₂ asphyxiation, spleens were aseptically removed and single cell suspensions were prepared as previously described (9). For the in vivo supplementation study, isolated splenocytes were used for assessment of proliferation (see below). For the in vitro supplementation studies, T cells were further purified from splenocytes using a Pan T cell isolation kit according to the manufacturer’s protocol (Miltenyi). Mitomycin (20 μg/mL, Sigma)-treated splenocytes isolated from BALB/c mice were used as antigen-presenting cells and were pulsed with OVA232–239 (0.3 μmol/L) in the presence or absence of EGCG (10 μmol/L) for 3 h. Antigen-presenting cells were washed to remove EGCG and then added to the CD4+ T cells from DO11.10 mice that had been preincubated with or without EGCG. After incubation for 72 h, cell proliferation was determined by [³H]-thymidine uptake as described above.

Antigen-specific T cell proliferation and coculture of T cells and antigen-presenting cells. DO11.10 mice are transgenic mice on BALB/c background. The majority of T cells in DO11.10 mice are naive CD4+ T cells, which predominantly express a T cell receptor (TCR) that recognizes ovalbumin (OVA)323–339 peptide. For the EGCG dose-response experiment, splenocytes isolated from DO11.10 mice were incubated with EGCG at 2.5–10 μmol/L in the presence of specific antigen OVA232–239 (0.3 μmol/L, synthesized by the Tufts University Core Facility Laboratory) for 72 h. To determine the specific effects of EGCG on T cells and antigen-presenting cells, CD4+ T cells were purified from lymph node and spleen cells of DO11.10 mice using a CD4+ T cell isolation kit according to the manufacturer’s protocol (Miltenyi). Mitomycin (20 μg/mL, Sigma)-treated splenocytes isolated from BALB/c mice were used as antigen-presenting cells and were pulsed with OVA232–239 (0.3 μmol/L) in the presence or absence of EGCG (10 μmol/L) for 3 h. Antigen-presenting cells were washed to remove EGCG and then added to the CD4+ T cells from DO11.10 mice that had been preincubated with or without EGCG. After incubation for 72 h, cell proliferation was determined by [³H]-thymidine uptake as described above.

Cell division analysis. Splenocytes or purified T cells in PBS were labeled with 1 μmol/L of carboxyfluorescein diacetate succimidyl ester (Molecular Probes) as previously described (9). The labeled cells at 2 × 10⁵/well in a 96-well round-bottom cell culture plate were stimulated for 72 h with immobilized anti-CD3ε mAb and anti-CD28 mAb (anti-CD3/CD28, 10 μg/mL each). Upon harvesting, cells were stained with either allophycocyanin (APC)-conjugated anti-CD3 (for splenocytes) or APC-conjugated anti-CD4 and phycoerythrin (PE)-conjugated anti-CD8 (for purified T cells). Gating was performed on the CD3⁺ population in spleen cells and on the CD4⁺ or CD8⁺ population in purified T cells. The total number of mitoses per 100 gated cells was calculated using the formula: number of mitoses = (X₁ – X₂)/X₄² for X₁, X₂, and X₄ as the percentage of cells that undergo n times of division.

Cell cycle analysis. Purified T cells (2 × 10⁵ cells/well) in a 96-well round-bottom plate were stimulated with immobilized anti-CD3/CD28 for 48 h. Cells were then fixed and stained with 20 μg/mL propidium iodide (Sigma) at 37°C for 15 min. Samples were immediately analyzed by a FACSCalibur flow cytometer.

Interleukin-2 intracellular staining. Purified T cells at 2 × 10⁶/well in a 24-well plate were stimulated with immobilized anti-CD3/CD28 for 6 h in the presence of Golgastop (BD Pharmingen) to block protein export. Cells were harvested and incubated with purified anti-CD16/32 (Fc block, BD Pharmingen) for 15 min and then incubated with APC-anti-CD4 (eBioscience) or APC-anti-CD8 (eBioscience) in a staining buffer containing 0.09% NaN₃ (Sigma) and 1% fetal bovine serum in PBS for 30 min. The cells were fixed and permeabilized using a Cytofix/ Cytoperm kit (BD Pharmingen). Cells were stained with PE-antilymphocyte cytoplasmic antigen (IL)-2 (BD Pharmingen) or PE-IgG2b κ isotype control (BD Pharmingen) for 30 min. The cells were then washed in Perm/Wash buffer (BD Pharmingen) and resuspended in staining buffer for immediate analysis on a FACSCalibur flow cytometer.
IL-2 receptor α (CD25) expression. Purified T cells at $2 \times 10^5$/well were stimulated with immobilized anti-CD3/CD28 in a 96-well round-bottom cell culture plate for 48 h for CD25. Upon harvesting, cells were washed and incubated with FC block for 5 min and then with fluorescein isothiocyanate-anti-CD4, PE-anti-CD8, and APC-anti-CD25 (all from eBioscience) for 30 min at 4°C. Cells were then washed and resuspended in staining buffer for immediate analysis on a FACS Calibur flow cytometer.

IL-2 measurement. Purified T cells at $2 \times 10^5$/well were stimulated with immobilized anti-CD3/CD28 in a 96-well round-bottom cell culture plate for 6, 12, 18, 24, or 48 h. Cell-free supernatants were collected and stored at −70°C for later analysis. IL-2 concentration in cultures was determined by ELISA according to the manufacturer’s instructions (BD Pharmingen).

Real-time PCR for IL-2 and IL-2R mRNA expression. Purified T cells at $1 \times 10^5$/well were stimulated with immobilized anti-CD3/CD28 in a 24-well flat-bottom cell culture plate. Cells were collected after 2, 4, or 6 h of activation and washed with PBS. Total RNA was extracted with an RNeasy mini kit (Qiagen) according to the manufacturer’s instructions. One microgram of total RNA was converted to double-stranded cDNA using Superscript III RT (Invitrogen). Primers for IL-2, IL-2 receptor containing α-subunit (IL-2Ra), and β-actin were designed using Primer Express version 2.0 (Applied Biosystems). The primer pairs used in real-time PCR were the following: IL-2, 5′-GAACGAGCCACAGAATGGA-3′, 5′-CTCATCATGGAATTTGGC-3′; IL-2Ra, 5′-GCAACTCCTGCAAACTCCG-3′, 5′-TGACAATCCTCTGACGTGA-3′; β-actin, 5′-AGATCTCGAGCGAGGATGTCG-3′, 5′-CCACAGGTATTCCATCCCAAGA-3′. β-Actin was used as an endogenous control. Primer amplification efficacy and specificity were verified for each set of primers. cDNA levels of the genes of interest were measured using power SYBR green master mix on real-time PCR 7300 (Applied Biosystems). mRNA levels of the tested genes relative to β-actin mRNA were determined using the $2^{-ΔΔCt}$ method and as fold induction.

Statistical analysis. All results were expressed as means ± SEM. Statistical analysis was conducted using SYSTAT 10 statistical software. Differences were determined using 1-way or 2-way ANOVA followed by Tukey’s HSD post hoc procedure, or by t test when only 1 variable with 1 condition was tested. Significance was set at $P < 0.05$.

Results

Dietary EGCG supplementation suppresses ex vivo T cell proliferation. Compared with the mice fed the control diet, mice fed 0.3% EGCG had a lower lymphocyte proliferation under almost all the stimulation conditions, but those fed the 0.15% EGCG diet did not differ from controls (Fig. 1). These results indicate that dietary EGCG supplementation is effective in increasing EGCG or its metabolites in splenocytes to the levels that are sufficient to affect T cell response in a manner similar to that seen in our in vitro studies. Furthermore, these results support the validity of using in vitro supplementation with EGCG in subsequent studies to determine the mechanisms underlying the suppressive effect of EGCG on T cell proliferation.

In vitro EGCG supplementation inhibits T cell division and cell cycle progression. After 72 h of TCR stimulation with anti-CD3/CD28, T cells in total splenocytes (Fig. 2A), purified total T cells (Fig. 2B), CD4+ T cells (Fig. 2C), and CD8+ T cells (Fig. 2D) underwent several divisions, which were inhibited by EGCG in a dose-dependent manner (Fig. 2, Supplemental Fig. 1 for the original results). Among the T cells, CD4+ cells were more sensitive to inhibitory effects of EGCG (Fig. 2). EGCG inhibited cell proliferation at as low as 2.5 μmol/L in CD4+ cells while a significant inhibition did not occur in CD8+ T cells until the EGCG concentration was increased to 15 μmol/L. Furthermore, compared to the purified T cells being used alone, the proliferation of T cells in the presence of total splenocytes was inhibited more dramatically by EGCG, suggesting that EGCG inhibits T cell proliferation not only by directly affecting T cells but also by indirectly affecting other cells, perhaps mainly accessory cells/antigen-presenting cells, present in the total splenocytes.

EGCG inhibits antigen-specific T cell proliferation mainly through a direct effect on T cells. To determine the relative contribution of T cells and antigen-presenting cells to the EGCG-induced effects, we used antigen-specific T cell response to OVA. EGCG treatment of splenocytes from DO11.10 mice dose-dependently inhibited their proliferative response to OVA (Fig. 3A). When incubating antigen-presenting cells and T cells separately, with or without EGCG (10 μmol/L), we found that EGCG treatment of either antigen-presenting cells or T cells reduced T cell proliferation, but EGCG treatment of T cells had a more suppressive effect than that caused by EGCG-treated antigen-presenting cells (60 vs. 28% inhibition for T cells and antigen-presenting cells, respectively). When both antigen-presenting cells and T cells were treated with EGCG, the inhibition mostly reflected a direct effect of EGCG on T cells (Fig. 3B).

In vitro EGCG supplementation inhibits cell cycle progression. Because the inhibition of cell division may result from a reduced capability of cells entering the division phase, we tested the effect of EGCG on cell cycle progression. Whereas the resting T cells remained mainly at the G0/G1 phase, anti-CD3/CD28-stimulated T cells had an increased entry into S/G2/M phases; consistent with the finding in cell division assay, EGCG dose-dependently reduced the progression of T cells into S/G2/M phases (Fig. 4, Supplemental Fig. 2).

Effect of EGCG on IL-2 production and intracellular IL-2 levels in T cells. To further determine the mechanism of EGCG-induced T cell suppression, we evaluated the effect of EGCG on the ability of T cells to produce IL-2, a T cell growth factor essential in regulating T cell activation, expansion, and function. Surprisingly, however, EGCG dose dependently
increased IL-2 levels in the T cell cultures after 48 h stimulation with anti-CD3/CD28 (Fig. 5A). A further time course study revealed that IL-2 levels in the T cell cultures were not altered by EGCG treatment at 6, 12, 18, and 24 h, but were higher at 48 h poststimulation (Fig. 5B). Intracellular IL-2 analysis by flow cytometry showed that whereas the proportion of IL-2+ cells was slightly but significantly larger in CD8+ cells and tended to be greater ($P = 0.085$) in the CD4+ population (Fig. 5C, Supplementary Fig. 3), IL-2 production per cell in both CD4+ and CD8+ cells was lower in EGCG-treated cells compared with the control cells (Fig. 5D). In accordance with the results of IL-2 secretion at earlier time points (6, 12, 18, and 24 h), further analysis of IL-2 mRNA expression using real-time PCR showed no difference between EGCG-treated and control cells (data not shown). Taken together, EGCG did not significantly affect IL-2 synthesis.

EGCG reduces CD25 (IL-2Rα) expression in T cells. Because IL-2 is required to interact with high affinity IL-2Rα to exert its biological effects, we next investigated whether the effect of EGCG is associated with altered IL-2R levels given that IL-2 synthesis is not significantly affected by EGCG. We found that TCR stimulation with anti-CD3/CD28 increased both the percentage of cells expressing CD25 (IL-2Rα) in total T cells (Fig. 6A), CD4+ T cells (Fig. 6B), and CD8+ T cells (Fig. 6C), and the expression level of CD25 per cell in total T cells (Fig. 6D), CD4+ T cells (Fig. 6E), and CD8+ T cells (Fig. 6F). A great majority of T cells expressed CD25 at 48 h and the proportion of CD25+ cells was not affected by EGCG except at the highest supplementation level (Fig. 6A–C). However, the level of CD25 expression per cell was significantly diminished by EGCG in a dose-dependent manner (Fig. 6D–F). We then assessed whether reduced CD25 by EGCG was controlled at a transcriptional level and found that EGCG did not affect IL-2Rα mRNA expression in response to stimulation with anti-CD3/CD28 (data not shown), suggesting that EGCG reduces IL-2Rα through post-transcriptional mechanisms.

Discussion
In this study, we show that both in vivo and in vitro supplementation with EGCG can reduce T cell proliferation. The concentrations of EGCG used in the in vitro experiments of the present study are achievable by oral intake of high doses of EGCG as demonstrated in previous studies (6–8). The observed inhibition of T cell proliferation in mice fed a 0.3% EGCG diet further suggests that dietary supplementation with EGCG could achieve in vivo levels that would be adequate to suppress T cell
function similar to that observed after in vitro treatment. Based on the average consumption of 3 g/d by a mouse, diets containing 3 g/kg EGCG will provide mice with a daily intake of 9 mg EGCG, or 360 mg/(kg BW × d) for a mouse of 25 g. When this dose is converted from mice consuming 12 kJ/d to humans consuming 2000 kJ/d by using isocaloric calculation (8,17), it will be roughly equivalent to 26 mg/(kg BW × d) in humans, or 1820 mg EGCG/d consumed by a 70-kg person. A cup of tea contains 150–180 mg EGCG; commercially available EGCG supplements contain up to 350 mg EGCG. Therefore, although these doses are not usually used, they are achievable by consuming high doses of supplements or by drinking large quantities (10 cups or 2 L/d) of tea.

After confirming the T cell-suppressive effect of EGCG in vivo, we further determined the mechanism for this effect of EGCG in a series of in vitro experiments. First, we compared the effect of EGCG on splenocytes, which contain a mixture of cells, with that of purified T cells, because other cells present in splenocytes, in particular accessory cells/antigen-presenting cells, are involved in the regulation of T cell proliferation and could contribute to the effect of EGCG (18–20). The observation that the inhibitory effect of EGCG on T cell proliferation was more pronounced in cultures of mixed cell populations (total splenocytes) compared with those of purified T cells suggests that EGCG may also suppress T cell proliferation indirectly by affecting other cell types. We utilized an antigen-specific T cell response model to determine the relative contribution of T cells (direct) and non-T cells (indirect) to the EGCG-induced effects. We observed that EGCG exerted its T cell suppressive effect mainly through a direct effect on T cells (Fig. 3).

Several studies have shown that EGCG can induce H2O2 production under in vitro conditions and that H2O2 contributes to EGCG-induced cytotoxicity. However, this mechanism could not explain the decrease in T cell proliferation in this study, because EGCG did not affect cell viability and apoptosis at the levels used (data not shown). Furthermore, we previously showed that EGCG supplementation at the levels used in this study did not increase H2O2 production and the addition of catalase did not affect T cell suppression caused by EGCG (9).

Because EGCG mainly reduced T cell proliferation through a direct effect on T cells, to further determine the underlying mechanisms, we focused on T cells. Upon activation, resting T cells enter the cell cycle to rapidly proliferate and differentiate into effector cells. Two sequential signals in T cells, i.e. the TCR signal and the IL-2/IL-2R signal, are essential to support optimal T cell response and proliferation. First, TCR engagement with peptides bound to MHC on the surface of antigen-presenting cells drives the cells to enter cell cycle from a quiescent state (21,22), to synthesize IL-2, and to express IL-2R (23,24). Ligation of IL-2 with its receptor provides the second signal for cell cycle progression and T cell expansion (25). Thus, to determine the mechanism of direct effect of EGCG on T cells, we focused on T cell division, cell cycle progression, and IL-2/IL-2R interaction. We found that EGCG suppressed T cell proliferation and decreased the capacity of T cells to progress through different cell cycle phases and to eventually divide. However, EGCG did not affect IL-2 levels in T cell cultures < 24 h, but more IL-2 was detected in the cultures at 48 h poststimulation. These data suggest that the suppression of T cell proliferation by EGCG is due to less availability of IL-2R, which, in turn, leads to reduced IL-2 internalization and utilization rather than decreased IL-2 synthesis. This is supported by our results showing

![FIGURE 4](https://academic.oup.com/jn/article-abstract/140/8/1509/4689017)

**FIGURE 4** In vitro EGCG supplementation inhibits plate-bound anti-CD3/CD28-stimulated T cell cycle progression. Values are means ± SEM, n = 4. Means without a common letter differ, \( P < 0.05 \).

![FIGURE 5](https://academic.oup.com/jn/article-abstract/140/8/1509/4689017)

**FIGURE 5** Effect of in vitro EGCG supplementation on levels of secreted and intracellular IL-2 in T cells. (A) EGCG dose-dependent change in IL-2 secretion from T cells stimulated by plate-bound anti-CD3/CD28. (B) Time course of IL-2 secretion from T cells stimulated by plate-bound anti-CD3/CD28 in the presence of EGCG. Percentage (C) and mean fluorescence intensity (MFI) (D) of IL-2 levels in IL-2+ cells. Values are means ± SEM, \( n = 8 (A \text{ and } B) \) or 4 (C and D). Means without a common letter differ, \( P < 0.05 \). *Different from the corresponding control, \( P < 0.01 \).
that intracellular IL-2 and IL-2 mRNA levels are not significantly affected by EGCG. Reduced IL-2R is sufficient to impair T cell response as demonstrated by previous studies. For example, IL-2R blockade was shown to inhibit T cell proliferation without affecting IL-2 production (26) and to cause a defect in IL-2R signaling (27). Thus, EGCG-induced reduction in IL-2R expression might provide a reasonable explanation for most of the insufficient/impaired T cell activities observed in this study.

EGCG can bind to cellular and microbial proteins (28). For example, EGCG has been reported to directly bind CD4 protein on peripheral blood CD4+ T cells and thus to prevent the binding of anti-CD4 antibody (29). Given this, it is possible that the observed lower IL-2Rα expression on EGCG-treated T cells might be a result of interference with detection of IL-2Rα resulting from the binding of EGCG to IL-2, IL-2Rα, or detecting antibodies during analysis. However, the decrease in T cell proliferation despite an increase in IL-2 level argues against this possibility.

Consistent with its inhibition of T cell division, EGCG dose dependently blocked cell cycle progression into S and G2/M phases. This observation on primary T cells agrees with and extends the previous studies showing that EGCG induced arrest of the cell cycle in the G1 phase, resulting in suppressed cell growth in normal and transformed human fibroblasts (30), immortalized cervical cells (31), and human epidermoid carcinoma cells (32). How EGCG induces cell cycle arrest in T cells needs further investigation. One possibility is that this effect of EGCG may be associated with a change in cell cycle regulatory proteins and the signaling pathways leading to their activation/inactivation. Reduced expression of IL-2R by EGCG in T cells is likely to impair IL-2/IL-2R signaling, which in turn, through corresponding downstream pathways, affects cell cycle regulatory proteins and results in inhibition of cell cycle division and progression. Alternatively, EGCG may suppress T cell activation and proliferation via its function as a proteasome inhibitor (30,33,34). Proteasome-mediated degradation is required for controlling T cell activation and proliferation through removal of transcription factor suppressor IκBα and the subsequent activation of NF-κB or degradation of major cell cycle regulatory proteins such as cyclin, and CDK inhibitors p21Waf1/Cip1 and p27Kip1 (35). Proteasome inhibitors have been demonstrated to inhibit T cell proliferation, induce cell cycle arrest, and inhibit IL-2Rα expression (35–37).

In conclusion, EGCG has a direct inhibitory effect on T cells. CD4+ T cells are more sensitive to this inhibition than CD8+ T cells. In addition, EGCG may inhibit accessory and antigen-presenting cell function, which further potentiates its inhibition of T cells. Our data indicate that EGCG inhibits proliferation of purified T cells by inducing cell cycle arrest, which may in turn be due to impaired IL-2/IL-2R signaling. Although the doses used in the current study are above those achieved by moderate intake of tea or supplement, these amounts are achievable by taking a high dose of supplements or drinking large quantities (10 cups or 2 L/d) of tea. Thus, caution should be taken in consuming large amounts of EGCG, in particular for those with compromised T cell function. On the other hand, however, because dysregulated or imbalanced function among T cell subsets is thought to be a key contributor to autoimmune diseases, EGCG consumption might be useful in reducing pathogenesis of some autoimmune disorders.

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