Epigallocatechin-3-Gallate Inhibits Expression of Receptors for T Cell Regulatory Cytokines and Their Downstream Signaling in Mouse CD4+ T Cells1–3

Junpeng Wang,4 Munkyong Pae,4,6 Simin Nikbin Meydani,4,5 and Dayong Wu4,*

4Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA; and 5Department of Pathology, Sackler Graduate School of Biochemical Science, Tufts University, Boston, MA

Abstract

We previously showed a suppressive effect of epigallocatechin-3-gallate (EGCG) on T cell cycling and expansion as well as a paradoxical effect on IL-2 levels (upregulating) and IL-2 receptor (IL-2Rα expression (downregulating). Thus, in the current study, we tested the hypothesis that EGCG affects T cell responses via impairing the IL-2/IL-2R signaling. We found that EGCG inhibited anti-CD3/CD28-induced proliferation of naïve CD4+ T cells from C57BL/6 mice. EGCG increased accumulation of IL-2 but inhibited expression of IL-2R, including all its subunits [IL-2Ra, IL-2/IL-15Rβ, and common γ chain (γc)]. Using phosphorylation of STAT5 as a marker, we further found that EGCG suppressed IL-2R downstream signaling. Because IL-2R subunits IL-2/IL-15Rβ and γc are shared with IL-15R and γc is shared with IL-7R, we suspected that EGCG might also influence the signaling of IL-15 and IL-7, the two key regulators in maintaining T cell homeostasis. Results showed that EGCG suppressed IL-15 and IL-7 signaling; further, EGCG not only inhibited the subunits in IL-15R and IL-7R shared with IL-2R, but also affected their proprietary α chains in a manner that aligns with an impaired signaling. Although IL-2, IL-15, and IL-7 have separate and distinctive roles in regulating T cells, all of them are critical for T cell survival, expansion, and differentiation. Thus, these findings indicate an involvement of T cell growth cytokines in EGCG-induced T cell suppression through downregulated expression of their receptors and downstream signaling. This implies a potential application in controlling dysregulated T cell functions such as those observed in autoimmune and inflammatory disorders. J. Nutr. 142: 566–571, 2012.

Introduction

T cells are a key component in adaptive immune responses. Although T cells are crucial for the host’s defense against invading pathogens and the surveillance for neoplasms within, they are also responsible for attacking self (autoimmunity) when their homeostasis is disturbed. IL-2 is an important cytokine affecting multiple aspects of T cell biology. In addition to being a major growth factor that regulates T cell clonal expansion, their development into effector cells, and their survival, it is also a key factor in maintaining the tolerance necessary to prevent autoimmunity. Produced predominantly by activated T cells, IL-2 functions by binding to IL-2Rαβγc, which in its high affinity form consists of three subunits: IL-2Ra (CD25), IL-2Rβ (CD122), and γc (CD132). Before IL-2 is bound to its receptors, the IL-2Rα subunit can bind to the β chain to form IL-2Ra/β heterodimer (1). After the interaction of IL-2 and IL-2Ra/β heterodimer, γc chain is recruited to the IL-2/IL-2R complex, which results in activation of Janus kinase 1/3 and subsequent phosphorylation and activation of STAT5 (2,3). IL-2Rα primarily functions to increase ligand-binding affinity, whereas IL-2Rβ and γc participate in both ligand binding and signal transduction. IL-2Ra is a proprietary chain specific to IL-2, whereas IL-2Rβ and γc are also part of the IL-15R, and γc is a common component in the receptors of IL-4, IL-7, IL-9, and IL-21 (4,5).

1 Supported by USDA National Institute of Food and Agriculture grant 2010-65200-20360 and USDA, Agriculture Research Service contract no. 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 USDA.

2 Author disclosures: J. Wang, M. Pae, S. N. Meydani, and D. Wu, no conflicts of interest.

3 Supplemental Figures 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

4 Present address: Cellular and Molecular Physiology Section, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02115.

5 To whom correspondence should be addressed. E-mail: dayong.wu@tufts.edu.

6 Present address: Cellular and Molecular Physiology Section, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02115.

* To whom correspondence should be addressed. E-mail: dayong.wu@tufts.edu.

7 Abbreviations used: EGCG, epigallocatechin-3-gallate; γc, γ chain; IL-2R, IL-2 receptor; IL-7R, IL-7 receptor; IL-15R, IL-15 receptor; p-STAT5, phosphorylated Signal Transducer and Activator of Transcription 5; TCR, T cell receptor; Th, T helper.
Nutritional modulation has been shown to be a promising approach to modulation of T cell functions by either enhancing their defense function or suppressing their overactive or misdirected response. Green tea is one such dietary factor. The main bioactive components in green tea are catechins, among which EGCG is the most abundant and biologically active (6). We have reported that in vivo and in vitro supplementation with EGCG dose dependently inhibits T cell proliferation (7,8). This effect of EGCG is mainly due to inhibition of cell cycle progression and subsequent division. Further, we have learned that CD4+ T cells are more responsive than CD8+ T cells to this inhibitory effect of EGCG (7). Because IL-2 is a major growth factor important for T cell expansion, we further investigated EGCG’s effect on IL-2 production as a possible mechanism for EGCG’s T cell-suppressing effect. We found that IL-2 levels in the cultures of T cells stimulated for ±24 h were not affected by EGCG, but they were increased in the 48-h cultures. Of note, EGCG did not affect IL-2 mRNA expression levels. Higher levels of IL-2 in the EGCG-treated cultures appeared to contradict the EGCG-induced suppression in T cell proliferation given the well-recognized role of IL-2 in promoting T cell expansion. We then further observed that EGCG dose dependently reduced IL-2Rα expression. Taken together, a possible interpretation of the seemingly paradoxical effects of EGCG on IL-2 and IL-2Rα expression is that EGCG might have reduced the IL-2 utilization due to a lowered availability of high affinity IL-2R, resulting in impaired IL-2/IL-2R signaling. The results from our previous studies raised several questions that begged further elucidation. First, because only the expression levels of IL-2Rs were measured in our previous studies, the impact of EGCG on other IL-2R subunits needs to be determined. This is important due to the fact that in the absence of IL-2Rα, IL-2Rβ, and γc form an intermediate affinity IL-2R, which responds to high levels of IL-2 to convey signals. Second, while a lower IL-2Rα expression is assumed to reduce high affinity IL-2R and thus IL-2/IL-2R signaling, this needs to be confirmed by conducting the appropriate experiments. Furthermore, the T cells used in our previous study were not deprived of the CD4+CD25+ T cell population, i.e., natural regulatory T cells. Because Treg cells are assumed to reduce high affinity IL-2R and thus IL-2/IL-2R interaction, we found that EGCG inhibited expression of both IL-2Rα and IL-2Rβ in naive CD4+ T cells (7). Consistent with our previous report (7), EGCG reduced IL-2Rα (CD25) expression (Fig. 1A). In addition, we found that EGCG inhibited expression of both IL-2Rα and IL-2Rβ.

**Materials and Methods**

**Animals.** Specific pathogen-free C57BL/6 female mice (6–8 wk) were purchased from Charles River. These mice were maintained at a constant temperature and humidity with a 12-h-light:dark cycle. Water and a nutritionally adequate nonpurified diet formulated for rodents (7012 Teklad LM-485, Harlan Teklad) (10) were consumed ad libitum. This commercial diet contains 19.1% crude protein, 5.8% fat, 44.3% carbohydrate, 4.6% crude fiber, and 13.7% neutral detergent fiber. This diet was adequate in all nutrients and met the NRC nutrition requirements (11). 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.

**Naive CD4+ T cell purification.** After mice were killed with CO2 asphyxiation, spleens were aseptically removed and single cell suspension was prepared. The naïve CD4+ T cells were purified using CD4+CD62L+ T Cell Isolation kit II according to the manufacturer’s protocol (Miltenyi Biotec). The purity of naïve CD4+ T cells was >95%. Purified naïve CD4+ T cells (2 × 10^6/L) were cultured in 24-well culture plates in RPMI 1640 medium supplemented with 5% FBS, 25 mmol/L HEPES, 2 mmol/L glutamine, 100 kU/L penicillin, and 100 mg/mL streptomycin (all from Gibco Invitrogen).

**T cell proliferation.** CD4+ T cell proliferation was determined using the [3H]-thymidine incorporation method. Purified naïve CD4+ T cells (2 × 10^6/L) were stimulated with immobilized anti-CD3 (5 mg/L) and soluble anti-CD28 (1 mg/L) in 96-well, round-bottom culture plates in the presence of EGCG (10 μmol/L) at 37°C for 72 h. This dose of EGCG was selected based on our previous study using multi-doses of EGCG and T cell proliferation (7,8). Cells were pulsed with 18.5 kBq [3H]-thymidine (Perkin Elmer) during the final 4 h of incubation. The cells were harvested onto glass fiber filter mats (Wallac) by a Tomtec harvester (Wallac), and cell proliferation was quantified as the amount of [3H]-thymidine incorporation into DNA as determined by liquid scintillation counting in a 1205 Betalplate counter (Wallac). Results are expressed as Bq.

**IL-2 production and receptor expression for IL-2, IL-15, and IL-7.** Naive CD4+ T cells (2 × 10^6/L) were stimulated in 24-well culture plates with immobilized anti-CD3 (5 mg/L) and soluble anti-CD28 (1 mg/L) in the presence of EGCG (10 μmol/L) at 37°C for 72 h. Culture supernatants were collected and measured for IL-2 concentrations using the ELISA method (all reagents were from BD Pharmingen). The cells were harvested and stained with fluorochrome-conjugated antibodies as previously described (7). The antibodies used and their sources were: anti-CD4 (GK1.5), anti-CD25 (PC6.2) for IL-2Rα, anti-CD122 (TM-β1) for IL-2/IL-15Rβ, and anti-CD127 (TMe2) for γc from BD Pharmingen; anti-CD215 (DNT1Ra) for IL-15Rα and anti-CD127 (eBioSB/199) for IL-7Ra from ebioscience. Flow cytometry was conducted using an Accuri C6 flow cytometer and acquired data were analyzed with FlowJo 7.6 software.

**p-STAT5 expression.** After being stimulated with anti-CD3 (5 mg/L) and soluble anti-CD28 (1 mg/L) in the presence of EGCG (10 μmol/L) in 24-well culture plates at 37°C for 72 h, naïve CD4+ T cells were washed and then cultured (2 × 10^6/L) in fresh RPMI 1640 medium without FBS at 37°C to rest overnight. After overnight rest, cells were washed again and fresh medium was added so that no cytokines at measurable levels were present in the cultures. Recombinant IL-2 (20 μg/L), IL-7 (10 μg/L), or IL-15 (1 μg/L) was then added to the cultures and incubated at 37°C in a water bath for 0, 3, 5, and 10 min. Cells were fixed with 3.7% paraformaldehyde at room temperature for 20 min. After being washed with ice-cold PBS, cells were suspended in 90% iced 70% ethanol and kept at −20°C for 2 h to allow for permeabilization. Finally, cells were washed and stained with anti-CD4 and anti-p-STAT5 (pY694, 47; BD Pharmingen) and subjected to flow cytometry analysis in an Accuri C6 flow cytometer. Data were analyzed using FlowJo 7.6 software.

**Statistical analysis.** All results were expressed as means ± SEM. Statistical analysis was conducted by unpaired Student’s t test or 2-way ANOVA followed by Tukey’s HSD post hoc procedure using Systat 12 statistical software. Significance was set at P < 0.05.

**Results**

**EGCG inhibits IL-2R expression and proliferative response in naïve CD4+ T cells.** Consistent with our previous report (7), EGCG reduced IL-2Rα (CD25) expression (Fig. 1A). In addition, we found that EGCG inhibited expression of both IL-2Rα and IL-2Rβ. The effect of EGCG on IL-2Rα expression is dose dependent as the level of IL-2Rα expression was decreased at 1 μmol/L EGCG (Fig. 1B). The expression levels of IL-2Rβ were also decreased at 1 μmol/L EGCG (Fig. 1B).

**p-STAT5 expression.** After being stimulated with anti-CD3 (5 mg/L) and soluble anti-CD28 (1 mg/L) in the presence of EGCG (10 μmol/L) in 24-well culture plates at 37°C for 72 h, naïve CD4+ T cells were washed and then cultured (2 × 10^6/L) in fresh RPMI 1640 medium without FBS at 37°C to rest overnight. After overnight rest, cells were washed again and fresh medium was added so that no cytokines at measurable levels were present in the cultures. Recombinant IL-2 (20 μg/L), IL-7 (10 μg/L), or IL-15 (1 μg/L) was then added to the cultures and incubated at 37°C in a water bath for 0, 3, 5, and 10 min. Cells were fixed with 3.7% paraformaldehyde at room temperature for 20 min. After being washed with ice-cold PBS, cells were suspended in 90% ice-cold methanol and kept at −20°C for 2 h to allow for permeabilization. Finally, cells were washed and stained with anti-CD4 and anti-p-STAT5 (pY694, 47; BD Pharmingen) and subjected to flow cytometry analysis in an Accuri C6 flow cytometer. Data were analyzed using FlowJo 7.6 software.

**Statistical analysis.** All results were expressed as means ± SEM. Statistical analysis was conducted by unpaired Student’s t test or 2-way ANOVA followed by Tukey’s HSD post hoc procedure using Systat 12 statistical software. Significance was set at P < 0.05.
Together with IL-2, regulate the homeostasis of T cells. Similar to what we observed with IL-2, the addition of IL-15 or IL-7 greatly induced formation of p-STAT5, which was inhibited by EGCG (Fig. 3A for IL-15; Fig. 3B for IL-7; Supplemental Fig. 2A,B).

**EGCG inhibits IL-15Ra expression and prevents activation-induced decline in IL-7Ra expression in naive CD4⁺ T cells.**

In addition to the subunits shared with IL-2R, IL-15R, and IL-7R, each has a proprietary subunit, i.e., IL-15Ra (CD215) and IL-7Ra (CD127), respectively. As expected and consistent with previous reports (12–14), IL-15Ra expression was increased, but high constitutive IL-7Ra expression was greatly reduced after TCR activation. EGCG treatment, however, reduced the TCR activation-induced increase in IL-15Ra expression (Fig. 4A; Supplemental Fig. 3A). Interestingly, EGCG partially prevented the TCR activation-induced reduction in IL-7Ra expression at 24 and 48 h but not at 72 h post-stimulation (Fig. 4B; Supplemental Fig. 3B). Unstimulated cells have a low IL-15Ra expression (~0.5%) and high IL-7Ra expression (~73%) regardless of incubation, which did not change over time and were not affected by EGCG (data not shown).

**Discussion**

We have previously shown a suppressive effect of EGCG on T cell cycling and expansion as well as a paradoxical effect on IL-2 levels (upregulating) and IL-2Ra expression (downregulating). In this study, we reproduced those findings using naive CD4⁺ T cells. Because natural regulatory T cells, known to inhibit T cell proliferation, were removed during the preparation of naive CD4⁺ T cells, these results represent a direct suppressive effect of EGCG on T cells rather than an indirect effect by altering the number and/or function of Treg cells.

IL-2 production and IL-2Ra expression depend greatly on TCR activation. Formation of high-affinity quaternary IL-2/IL-2R complex triggers signal transduction, after which the IL-2/IL-2R complex is rapidly internalized. The fate of the components in the complex differs: IL-2, IL-2Rβ, and γc are quickly degraded, but IL-2Ra is recycled to the cell surface (15); IL-2Ra is absent on naive or memory T cells, but it is induced after TCR stimulation; IL-2Rβ is constitutively expressed by natural killer cells, natural killer T cells, and memory CD8⁺ T cells, but it is also induced on naive T cells; and γc is constitutively expressed by all lymphoid cells (15). In our previous studies, we reported an inhibitory effect of EGCG on IL-2Ra, the propri-

**FIGURE 1** EGCG inhibits IL-2R expression (A) and proliferative response (B) and causes greater accumulation of IL-2 (C) in naive CD4⁺ T cells stimulated with anti-CD3/anti-CD28 in the presence of EGCG (10 μmol/L). Values are mean ± SEM, n = 3. Within + or −EGCG, means without a common letter differ, P < 0.05. Asterisks indicate different from −EGCG: * P < 0.05, ** P < 0.01, and *** P < 0.001 for each marker or at a time. EGCG, epigallocatechin-3-gallate.

**FIGURE 2** EGCG inhibits IL-2-induced STAT5 phosphorylation of naive CD4⁺ T cells stimulated with anti-CD3/anti-CD28 in the presence of EGCG (10 μmol/L). Values are mean ± SEM, n = 3. *Different from −EGCG at that time, P < 0.05. EGCG, epigallocatechin-3-gallate.

**EGCG inhibits IL-2R downstream signaling in naive CD4⁺ T cells.** Because IL-2 is required to interact with high-affinity IL-2R to exert its biological effects, we next investigated whether EGCG-induced low expression of IL-2R would result in an impaired IL-2/IL-2R signaling. We found that phosphorylation of STAT5, a downstream indicator for IL-2/IL-2R signaling, was reduced in EGCG-treated cells (Fig. 2; Supplemental Fig. 1).

**EGCG also inhibits IL-15 and IL-7 downstream signaling in naive CD4⁺ T cells.** We further investigated whether reduced expression of IL-2Rβ and γc by EGCG affects the downstream signaling of IL-15 and IL-7, which share subunits of IL-2R, and
that EGCG also inhibited expression of two other subunits of IL-2R: IL-2Rβ and γc can form an intermediate affinity receptor that is sufficient for IL-2R signaling in the presence of high levels of IL-2 (17,18). In this study, we expanded our previous findings by showing that EGCG inhibited expression of all three IL-2R subunits on activated naïve CD4+ T cells, wherein IL-2Rβ and γc are shared with IL-15Rα and IL-7Rα, respectively (13,29). However, in contrast to IL-7, the key regulators known to control T cell survival and proliferation (12,13,16,28,29), both IL-15 and IL-7 signaling were partly blocked by EGCG as indicated by the reduced phosphorylation of STAT5. Our further investigation on the involvement of the proprietary α chains for IL-15R and IL-7R in the EGCG-induced inhibition revealed that similar to the case for IL-2, EGCG inhibited IL-15Rα expression induced by TCR activation. Thus, blocked IL-15 signaling can be attributed to inhibited expression of all subunits in the IL-15R complex by EGCG. Regarding IL-7R expression in this context, the situation appears a little more complex and perplexing. The binding of IL-7 to its receptor triggers activation of downstream signaling, particularly the one via STAT5. Certainly, future work is needed to more directly determine the role of STAT5 in EGCG-induced impairment of T cell activation and proliferation.

Given that EGCG inhibits expression of all three IL-2R subunits on activated naïve CD4+ T cells, wherein IL-2Rβ and γc are shared with IL-15Rα and IL-7Rα, we suspected that EGCG might also influence the signaling of IL-15 and IL-7, the key regulators known to control T cell survival and proliferation (12,13,16,28,29). Indeed, both IL-15 and IL-7 signaling were partly blocked by EGCG as indicated by the reduced phosphorylation of STAT5. Our further investigation on the involvement of the proprietary α chains for IL-15R and IL-7R in the EGCG-induced inhibition revealed that similar to the case for IL-2, EGCG inhibited IL-15Rα expression induced by TCR activation. Thus, blocked IL-15 signaling can be attributed to inhibited expression of all subunits in the IL-15R complex by EGCG. Regarding IL-7R expression in this context, the situation appears a little more complex and perplexing. The binding of IL-7 to its receptor triggers activation of downstream signaling pathways that are responsible for maintaining T cell survival and driving T cell proliferation (13,29). However, in contrast to other γc cytokines, for which T cell activation and/or ligand binding upregulate expression of respective cytokine receptor by T cells, IL-7Rα is constitutively expressed by resting T cells but is downregulated by its ligand (IL-7) (14) or in response to various stimuli, including TCR activation (30) and other pro-survival cytokines (14). Although the significance of this distinct

FIGURE 3 EGCG inhibits IL-15 (A) or IL-7 (B) induced STAT5 phosphorylation of naïve CD4+ T cells stimulated with anti-CD3/anti-CD28 in the presence of EGCG (10 μmol/L). Values are mean ± SEM, n = 3. *Different from −EGCG at that time, P < 0.05. EGCG, epigallocatechin-3-gallate.

FIGURE 4 Effect of EGCG on IL-15Rα (A) and IL-7Rα (B) expression of naïve CD4+ T cells stimulated with anti-CD3/anti-CD28 in the presence of EGCG (10 μmol/L). Values are mean ± SEM, n = 3. Asterisks indicate different from −EGCG at that time: * P < 0.05, ** P < 0.01. EGCG, epigallocatechin-3-gallate; IL-7R, IL-7 receptor; IL-15R, IL-15 receptor.
pattern regulating IL-7R expression remains unclear, it has been suggested that because naïve T cells must be signaled by IL-7 to survive and because IL-7 is limited, the reduced IL-7R expression on T cells that have received cytokine-mediated survival signals would prevent these cells from competing with un-signalized T cells for the remaining IL-7 (14). Thus, our data suggest that the hindered disappearance of IL-7Rα by EGCG would cause a reduced availability of IL-7, leading to impaired naïve T cell survival/expansion and their differentiation to effector and memory T cells. The observation that EGCG reduces IL-7Rα disappearance could have implications for limiting autoimmunity.

In the current study, we focused on determining the effect of EGCG on IL-2R expression and its downstream signaling. However, the finding that EGCG inhibited expression of all three IL-2R subunits, some of which are shared by IL-15 and IL-7, led us to further investigate their signaling pathway. We found that EGCG not only inhibited the subunits in IL-15R and IL-7R that are shared with IL-2R, but it also affected their proprietary α chains in a manner that aligns with an impaired T cell activation signaling. Although IL-2, IL-15, and IL-7 each have distinct roles in regulating T cells, they all make critical contributions to T cell survival, expansion, and differentiation. Thus, the inhibited expression of their receptors and downstream signaling by EGCG provides a mechanistic explanation for the EGCG-induced suppression of T cell expansion and their effector functions. Indeed, we have reported that EGCG inhibited T cell proliferation and IFNγ production induced by TCR activation (7,8). Although this effect of EGCG may not be desirable for the normal T cell immune response critical for the body’s defense against pathogens, it may be utilized to hinder a dysregulated T cell response, as in the case of autoimmune disease. Pertinent to this issue, we recently showed that dietary supplementation with EGCG ameliorated experimental autoimmune encephalomyelitis in mice, which was accompanied by an inhibited proliferation of autoreactive T cells and their differentiation into proinflammatory phenotypes, i.e., Th1 and Th17 (31).

The concentration (10 μmol/L) of EGCG used in the current study is considered to be within a physiologically relevant range, i.e., it can be achieved if high doses of pure EGCG are taken, as suggested by previous studies (32,33). We previously showed that in vitro EGCG supplementation at concentrations of 2.5–10 μmol/L dose-dependently inhibits T cell proliferation and cell cycle progression (7,8). Furthermore, oral administration of EGCG in doses of 0.3 and 0.6% was effective in suppressing T cell proliferation as well as attenuating experimental autoimmune encephalomyelitis (7,31).

In summary, we demonstrated in this study that EGCG has an inhibitory effect on receptor expression of important T cell growth cytokines, which are associated with a reduction in their corresponding downstream signaling activities. These results suggest a potential mechanism for EGCG-induced suppression of T cell’s ability to expand and function. Future studies are needed to determine how EGCG affects cytokine receptor expression, e.g., at gene expression or post-translational level.

Acknowledgments
The authors thank Stephanie Marco for her help in the preparation of the manuscript. J.W., D.W., and S.N.M. designed the research; J.W., M.P., and D.W. conducted research; J.W. and D.W. analyzed data; J.W., D.W., and S.N.M. wrote the paper; and D.W. had primary responsibility for final content. All authors have read and approved the final manuscript.

Literature Cited
2. Grant AJ, Roesler E, Ju G, Tsudo M, Sugamura K, Waldmann TA. The interleukin 2 receptor (IL-2R): the IL-2Rα subunit alters the function of the IL-2Rβ subunit by enhancing IL-2 binding and signaling by mechanisms that do not require binding of IL-2 to IL-2Rα subunit. Proc Natl Acad Sci USA. 1992;89:2165–9.


32. Lambert JD, Lee MJ, Diamond L, Ju J, Hong J, Rose M, Newmark HL, Yang GS. Dose-dependent levels of epigallocatechin-3-gallate in human colon cancer cells and mouse plasma and tissues. Drug Metab Dispos. 2006;34:8–11.


Green tea epigallocatechin-3-gallate and T cell regulatory cytokines