Green tea EGCG, T-cell function, and T-cell-mediated autoimmune encephalomyelitis

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ABSTRACT

Autoimmune diseases are common, disabling immune disorders affecting millions of people. Recent studies indicate that dysregulated balance of different $CD4^+$ T-cell subpopulations plays a key role in immune pathogenesis of several major autoimmune diseases. Green tea and its active ingredient, epigallocatechin-3-gallate (EGCG), have been shown to modulate immune cell functions and improve some autoimmune diseases in animal models. In a series of studies we determined EGCG's effect on T-cell functions and its application in autoimmune diseases. We first observed that EGCG inhibited $CD4^+$ T-cell expansion induced by polyclonal (mitogens or anti-CD3/CD28) or antigen-specific stimulation. We then showed that EGCG suppressed expansion and cell cycle progression of naïve $CD4^+$ T by modulating cell cycle-related proteins. EGCG also inhibited naive $CD4^+$ T-cell differentiation into Th1 and Th17 effector subsets by impacting their respective signaling transducers and transcription factors. These results suggest that EGCG may improve T-cell-mediated autoimmune diseases. Using the experimental autoimmune encephalomyelitis (EAE) mice, an animal model for human multiple sclerosis, we found that dietary supplementation with EGCG attenuated the disease's symptoms and pathology. These EGCG-induced changes are associated with findings in the immune and inflammation profiles in lymphoid tissues and the central nervous system: a reduction in proliferation of autoreactive T cells, production of proinflammatory cytokines, and Th1 and Th17 subpopulations, and an increase in regulatory T-cell populations. These results suggest that green tea or its active components may have a preventive and therapeutic potential in dealing with T-cell-mediated autoimmune diseases. However, the translational value of these findings needs to be validated in future human studies.

INTRODUCTION

Autoimmune disease is characterized by dysregulated immune and inflammatory responses, which attack self-tissue and cause loss of function. Its etiology is still unclear. Genetic predisposition is a key risk factor; however, environmental factors are also involved. The common therapies for autoimmune disease have limited efficacy and often come with serious side effects. Natural products as a modifiable environmental factor have been studied for their potential role in developing the preventive and therapeutic strategies for autoimmune disorders. The most commonly used such natural products include nutrients, such as certain vitamins and lipids, as well as non-nutrient phytochemicals, particularly a variety of phenolic compounds. Among these phytochemicals, tea and tea-derived catechins appear to stand out to be promising candidates in this regard, which is the topic discussed in detail in this review, but mainly exemplified by the experimental evidence generated from the author's laboratory.

Tea is the most commonly consumed beverage worldwide, and green tea takes a portion about 20%. Green tea contains high amounts of catechins, accounting for 10–15% of its dry weight. The major catechins in green tea are epicatechin, epicatechin-3-gallate, epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG). Among them, EGCG is the most abundant and most biologically active, accounting for 50–80% of total tea catechins; thus, EGCG is the tea catechin that has been predominantly used in green tea research. Studies have suggested that green tea consumption may offer health benefits in terms of preventing or mitigating the development of various diseases such as cardiovascular,[1] autoimmune,[2–6] inflammatory,[7 8] neurodegenerative diseases,[9] and certain cancers[10–13] as well as obesity.[14–16] These reported effects of green tea are thought to be mainly due to the catechins it contains, in particular EGCG. Green tea and EGCG have been shown to be capable of modulating several aspects of innate and adaptive immunity.[17] Studies have demonstrated evidence supporting the role of EGCG in modulating immune cell functions, particularly T-cell-related activities including T-cell activation, proliferation, and differentiation into different subpopulations of effector cells. These results have shed light on the potential application of EGCG in improving immunodysregulation and related diseases. This paper will provide an overview of the research conducted in the author's laboratory regarding EGCG's effect on T-cell functions and T-cell-mediated autoimmune disease. Many other investigators' works are cited for the purpose of providing adequate background to facilitate discussion; however, it is not the author's intention to give an extensive review of the literature on this subject.

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EGCG AND T-CELL-MEDIATED FUNCTIONS

T-cell proliferation

The ability of T cells to proliferate in response to stimulation has been widely used as an indicator for assessing the overall immune competence of an individual. Thus, the assay for T-cell proliferation was the most common method used to define the immunomodulatory effects of bioactive dietary components including green tea/EGCG. The effects of EGCG on T-cell functions were noted in the early studies as demonstrated largely by the altered lymphocyte proliferation after in vitro supplementation with tea extracts or catechins such as EGCG. A study by Hu *et al*[18] reported that EGCG at a dose of 24 μg/mL (55 μM) and higher inhibited proliferation of B and T cells, but more so in T cells. Similarly, the later studies reported that in vitro supplementation with green tea extracts inhibited lymphocyte proliferation induced by T-cell mitogen concanavalin A (Con A)[19] or allogeneic stimulator cells.[19 20] While the data obtained from these in vitro studies have provided useful information guiding us to develop the future studies, a major concern is that in most if not all of these studies, the investigators have used high doses of EGCG way above the physiologically relevant levels, that is, those achievable in the body tissues should it be orally administered, making it questionable to extrapolate these results to in vivo conditions or its potential application in clinical setting.

To address this issue, first it is necessary to use physiologically relevant concentrations of EGCG (<10 μM) in the in vitro study. For this purpose, we conducted a study using EGCG at concentrations 2.5–10 μM and showed that EGCG dose-dependently inhibited Con A-stimulated splenocyte proliferation, T-cell division, and cell cycle progression of T cells.[21] We also demonstrated that this effect of EGCG was not due to apoptosis nor was it related to the reported EGCG-induced hydrogen peroxide generation since we found no difference in hydrogen peroxide generation with the concentrations of EGCG used; furthermore, adding catalase to cultures to degrade hydrogen peroxide did not prevent EGCG-induced inhibition of T-cell response.[21] In this study, T-cell proliferation took place in the presence of total splenocytes, which are composed of multiple cell types. Given this, we were still unable to convincingly conclude that EGCG has a direct effect on T cells. Therefore, in a subsequent study, we investigated the direct effect of EGCG (2.5–15 μM) on purified T cells, so that we would be able to rule out indirect effects via accessory cells and as such, to determine whether and how EGCG directly inhibits T cells.[22] After more definitive experiments with in vitro EGCG supplementation, we learned that EGCG inhibited anti-CD3/CD28-stimulated cell division in $CD4^+$ and $CD8^+$ T cells but more so for the former. Since anti-CD3/CD28 or Con A represents a polyclonal T-cell receptor (TCR) stimulation that induces a universal T-cell response, we conducted further experiments to mimic natural antigen-specific, T-cell-mediated immune response to determine EGCG's effect. In a model in which ovalbumin served as an antigen to induce antigen-specific T-cell proliferation after processed by antigen-presenting cells (APCs), we added EGCG (10 μM) to pretreat either or both of purified T cells and APCs. We found that T-cell proliferation could be inhibited by EGCG treatment of either T cells or APCs, with largest inhibition being found in the co-cultures of T cells and APCs pretreated with EGCG; however, the direct effect of EGCG on T cells contributed more to the final inhibition of T-cell proliferation.[22]

Owing to the fact that factors such as bioavailability are complicated, dynamic environment in the body do not apply to cell-based in vitro assays; the key findings obtained from the in vitro experiments need to be validated in the in vivo study, which would help determine whether the observed effect of EGCG is meaningful and has potential application. Thus, we fed mice a diet containing 0.3% EGCG for 6 weeks and found that consistent with the in vitro study, EGCG was effective at reducing ex vivo T-cell proliferation triggered by either Con A or anti-CD3/CD28. These results confirm that dietary EGCG supplementation could allow its distribution in the body that would be sufficient to inhibit T-cell function.[22]

T-cell cytokines, cytokine receptors, and their downstream signaling

Another important indicator for assessing T-cell function is the T-cell's ability to generate cytokines. These T-cell-derived cytokines are critical in supporting and regulating T-cell expansion, differentiation, and effector functions as well as in regulating the functions of other immune cells, particularly macrophages and B cells. Studies have suggested that green tea and EGCG may impact T-cell cytokine production and expression of cytokine receptors leading to changes in the corresponding downstream signaling within T cells. Along this line, in vitro EGCG treatment is shown to inhibit T-cell cytokine production under different conditions: interleukin (IL)-2 production in the murine mixed lymphocyte reactions,[20] IL-2, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ production in bacterial superantigens-stimulated peripheral blood mononuclear cells (PBMC),[23] and IFN-γ production in Con A-stimulated murine splenocytes,[21] or in anti-CD3/CD28-stimulated purified murine T cells and $CD4^+$ T cells.[24] However, other investigators have reported different results. For example, in vitro EGCG supplementation is shown to increase IL-2 production induced by phorbol 12-myristate 13-acetate (PMA)/phytohemagglutinin (PHA) stimulation in human PBMC.[25] These controversial results may be related to the difference in culture systems including cell type, EGCG concentration, stimulation agent, and incubation time, among other things. Sometimes a time course study helps for defining the dynamic change in cytokine production responding to EGCG treatment. For example, we found that EGCG had no effect on IL-2 concentrations in the cultures of T cells stimulated for <24 hours but it dose-dependently increased IL-2 concentrations in the 48 hours culture (EGCG at 2.5–15 μM).[22] Since we found no change in the intracellular IL-2 levels and its mRNA expression,[22] it does not seem likely that EGCG had any effect on IL-2 synthesis. However, we found an EGCG-induced reduction in IL-2 receptor (IL-2R) expression, which suggests that more IL-2 detected might reflect an increased IL-2 accumulation, possibly due to a reduced IL-2 internalization and usage. We have further demonstrated that all three IL-2R subunits (α, β, γc) are affected by EGCG.[22 26] Decreased IL-2R expression is expected to impair the IL-2R signaling. Indeed, we found

that phosphorylation of signal transducers and activators of transcription (STAT)5, an indicator of IL-2R downstream activation, was reduced after naïve $CD4^+$ T cells were treated with EGCG.[26] Given the role of IL-2 as a prominent T-cell growth factor, and previously reported correlation between IL-2-induced T-cell proliferation and STAT5 activation,[27 28] we believe that decreased IL-2R expression and the resulting mitigation of its downstream signaling is an important mechanism for the EGCG's inhibitory effect on T-cell proliferation. Since EGCG inhibits expression of all IL-2R subunits and some of them also serve as receptors for IL-15R (IL-2Rβ, γc) and IL-7R (γc), we speculated that EGCG might also affect signaling of these two cytokines. Indeed, we confirmed that both IL-15 and IL-7 downstream signaling were partly blocked by EGCG.[26] IL-15 and IL-7 are important in regulating T-cell survival, expansion, and differentiation.[29–32] Thus, reduced expression of their receptors and resulting mitigation in the downstream signaling by EGCG may represent another mechanistic aspect of EGCG-induced T-cell suppression.

T-cell activation and cell cycle progression

T-cell activation can be triggered by engagement of TCRs with peptide antigens presented by the major histocompatibility complex on APCs, a process called TCR signaling which culminates in IL-2 production and T-cell expansion. EGCG may inhibit cytokine production and T-cell proliferation by interfering with the early signaling events in T-cell activation. A previous study reported that EGCG could inhibit the early T-cell signaling events in Jurkat T cells, such as activation of Zap70, linker for activation of T cells (LAT), phospholipase Cγ1, extracellular signal-regulated kinases (ERK), mitogen-activated protein kinases (MAPK) activity, and nuclear transcription factor AP-1.[33] After TCR activation, both the TCR and IL-2/IL-2R signals drive T cells to enter the cell cycle. Therefore, by inhibiting these signals in T cells, EGCG may interrupt cell cycle progression. This is proved to be true as we found that EGCG mitigated the T-cell cycle progression as measured in splenocytes[21] or purified T cells.[22] In cell cycle, traverse of G0/G1 and entry into S phase are known to be controlled by cyclin-dependent kinases (CDKs).[34] CDK activity is promoted by cyclins and inhibited by CDK inhibitors. As a potent CDK inhibitor, p27kip1 functions to maintain the cells in a quiescent state.[35] It has been shown that IL-2 induces T-cell cycle progression by inhibiting p27kip1,[36 37] while EGCG induces cell cycle arrest by increasing p27kip1 in human natural killer cells (NK) and T-cell lines.[38 39] In a recent study,[40] we found that EGCG inhibited anti-CD3/CD28-stimulated naïve $CD4^+$ T-cell division and expression of cell proliferation marker Ki-67. Cell cycle analysis showed that stimulation triggered cell cycle progression resulting in a reduced proportion of cells in G0/G1 and increased progression into S/G2/M phases. This stimulation-induced process was impeded by EGCG as evidenced by increased cells arrested in G0/G1 phase by 45%, decreased DNA synthesis activity in S phase by 51%, and decreased progression into G2/M phage by 14%. We further showed that EGCG reduced the expression of cell cycle-related proteins cyclin A, B, and D. Consistent with this, EGCG also inhibited expression of CDK 2, 4, 5, and 6. In addition, EGCG prevented activation-induced phosphorylation (inactivation) of the cell cycle suppressor retinoblastoma protein and reduction in CDK inhibitor p27Kip1 expression. Together, these results suggest that EGCG's inhibitory effect on $CD4^+$ T-cell division may be due to impeded cell cycle progression, which in turn, is related to altered activity and/or expression of several molecules known to regulate cell cycle progression.

$CD4^+$ T-cell differentiation

$CD4^+$ T cells are critical for the body's defense by their 'helper' function, including the essential role in facilitating B-cell function and activating the cells in the innate immune system. On the other hand, differentiated $CD4^+$ T cells of several subtypes are also known to be key regulators in the pathogenesis of T-cell-mediated autoimmune diseases. Several subsets of $CD4^+$ T cells have been identified, mainly based on predominant production of their respective hallmark cytokines. Among them, Th1 cells, which predominantly produce IFN-γ, IL-2, and TNF-α, can activate macrophages and help them to destroy ingested microbes, thus are primarily responsible for elimination of intracellular pathogens. Th2 cells, which mainly produce IL-4, IL-5, IL-10, and IL-13, can help B cells to produce antibodies and consequently, they help eliminate extracellular pathogens. The later identified Th17 cells based on their production of IL-17/IL-22 are a prominent effector lineage with proinflammatory actions. This subset of $CD4^+$ T cells are responsible for defense against the extracellular bacteria and fungi, in particular for those not well covered by Th1 or Th2 immunity.[41–43] Regulatory T cells (Treg) are a group of T cells defined by surface expression of CD4 and CD25, together with the presence of transcription factor forkhead box P3 (Foxp3). Treg cells are a central player in maintaining self-tolerance as well as limiting excessive immune responses.[44] Treg cells are a main producer of IL-10 and transforming growth factor (TGF)-β. Recently, investigators have suggested the presence of a distinguished subset of $CD4^+$ T cells, namely Th9 cells, which produce IL-9 and promote Th17 development.[45–48] Studies have come to a general consensus that upregulated function of Th1, Th9, Th17 cells and/or suppressed function of Treg cells are key factors in pathogenesis of T-cell-mediated autoimmune diseases; Th2 cells are largely involved in allergies and asthma.

Taking advantage of the recent progress in the research of $CD4^+$ T-cell biology, we further expanded our research on EGCG's T-cell-modulating effect by determining how EGCG impacts development of different $CD4^+$ T-cell lineages, which would allow us to better understand EGCG's potential role in the immunopathology of autoimmune diseases. We first observed that EGCG dose-dependently (2.5–15 μM) inhibited IFN-γ production while causing little change in IL-4 and IL-10 production by T cells.[24] These results indicate that EGCG may specifically suppress Th1 response without affecting Th2 response, suggesting that EGCG may differentially impact the response of T-cell subsets rather than universally affecting all T cells. In a subsequent study, in which $CD4^+$ T cells were polarized under specific conditions to become Th1, Th2, Th9, Th17, or Treg lineages in the presence of 10 μM EGCG, we found that EGCG impeded the differentiation of naive $CD4^+$ T cells into Th1, Th9, and Th17

subsets and that it also partly prevented IL-6-induced suppression of Treg development.[49] Consistent with the previous results of Th2 response as assessed by IL-4 and IL-10 production, EGCG had no effect on Th2 differentiation. To support these results from a mechanistic aspect, we further showed that EGCG suppressed expression of T-bet, PU.1, and RORγt, the specific transcription factors for Th1, Th9, and Th17 differentiation, respectively. These changes were also found to be largely paralleled to the EGCG-induced suppression in the expression of several signal transducers including p-STAT1 and p-STAT3 for Th1 and Th17, respectively. In a study by Wong *et al*,[50] in vitro EGCG treatment (2 and 10 μM) induced Foxp3 and IL-10 mRNA expression in Jurkat T cells, and mice receiving in vivo administration of EGCG (50 mg/kg, intraperitoneally daily for 7 days) had more Treg cells in their spleen and lymph nodes (LNs). Together, it seems that EGCG may inhibit proinflammatory $CD4^+$ T-cell subsets while maintaining/promoting anti-inflammatory Treg development; these effects of EGCG may be mediated by modulating the respective transducer proteins and transcription factors involved in the process of $CD4^+$ T-cell differentiation. Given all this, EGCG may represent a promising potential for improving T-cell-mediated autoimmune diseases.

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS, A PROTOTYPE OF T-CELL-MEDIATED AUTOIMMUNE DISEASES

Dysregulated T-cell responses play a key role in autoimmune pathology, which is responsible for the initiation and development of several T-cell-mediated, autoimmune diseases. Multiple sclerosis (MS) is prototypical of such disorders. MS is a T-cell-dependent, autoimmune inflammatory disease of the central nervous system (CNS) with the pathological hallmarks of inflammation, demyelination (loss of myelin sheaths insulating axons), axonal injury, and gliosis.[51] Experimental autoimmune encephalomyelitis (EAE) is a highly reproducible rodent model for human MS, and its immunopathology is well characterized. Thus, EAE is not only the most commonly used model for the research of human MS but also an appropriate tool to advance our understanding of T-cell-mediated autoimmunity. EAE can be induced by immunizing susceptible animals with a component of myelin such as myelin oligodendrocyte glycoprotein (MOG) or a peptide derived from it such as MOG peptide$_{35\text{-}55}$, emulsified in an adjuvant to generate myelin-reactive T cells. In a typical EAE model, animals develop perivascular inflammatory lesions and demyelination in the CNS, and consequently manifested symptoms, that is, paralysis of the tail, hind limbs, and forearms.

The pathogenesis of MS and EAE is still not completely understood, but the disease development is thought to be a process involving the autoreactive myelin-specific $CD4^+$ T cells that specifically target myelin sheath in the CNS resulting in tissue destruction and consequently, the loss of function.[52–54] For a long time, EAE is viewed as a Th1-mediated autoimmune disorder associated with increased activity of myelin-specific, IL-12-induced, and IFN-γ-producing $CD4^+$ Th1 cells. The evidence includes observations that Th1-specific cytokines are found high in active EAE lesions but decrease during remission,[55 56] transfer of myelin-Ag-specific T cells with a Th1 phenotype to healthy animals could induce EAE, and mice deficient in the transcription factors involved in Th1 differentiation were resistant to EAE development.[52 54–57] However, studies in the past decade have made significant progress in this area of research, and compelling evidence has accumulated to challenge the simple Th1-EAE paradigm. In particular, some of the autoimmune responses formerly believed to be caused by activation of Th1 cells are now directed to Th17 cells as a main culprit. It is evident that both Th1 and Th17 cells are key players in T-cell-mediated autoimmune diseases. While their relative importance in this regard is yet to be clearly defined, it appears that each has its own specificities in the pathological process of EAE.[58 59] For example, transfer of purified myelin-reactive Th1 cells to normal recipient animals was shown to be highly pathogenic and their homing to the CNS and resulting inflammation promoted Th17 cell infiltration.[60] In another study, adoptive transfer of either Th1 or Th17 cells was sufficient to induce mouse EAE; the mice showed similar symptoms but had distinct patterns of infiltrating innate immune cells: macrophages were dominant with Th1 transfer whereas neutrophils were dominant with Th17 transfer.[59] Another effector $CD4^+$ T-cell subset, Th9 cells, which are yet to be fully recognized as a specific population, has been implicated in MS pathogenesis based on the finding that adoptive transfer of Th9 cells into naïve recipients can induce EAE.[61] Treg cells is another important type of T cells in T-cell regulation and its crucial role as counter in T-cell-mediated autoimmune diseases is well summarized in the recent reviews.[62 63] Treg cells are well characterized for their function to maintain self-tolerance and to limit excessive immune responses,[44] and thus as expected, downregulated Treg cells either in number or in function are suggested to contribute to the development of autoimmune diseases.[62] Suppressive effect of Treg cells may be mediated through direct contact, secretion of cytokines such as TGF-β and IL-10, and competition for growth factors.[64] Increased symptom severity and mortality were observed in the EAE model after Treg cells were depleted with anti-CD25 Ab;[65] conversely, adoptive transfer of Treg cells could reduce the incidence of EAE.[66] Interaction between Treg and Th17 cells is noted not only for their counteractive role in the autoimmune process but also for the fact that Treg cells can be reprogrammed to develop into Th17 cells in the presence of TGF-β and IL-6. Taken together, the current research suggests that a dysregulated balance in $CD4^+$ T-cell differentiation contributes to the initiation and development of EAE. It is thus anticipated that if an agent could help restore and maintain the balance among different $CD4^+$ T cells, it may potentially have preventive and therapeutic value in MS as well as other T-cell-mediated autoimmune diseases.

EFFECT OF EGCG ON EAE

The first study demonstrating the effect of EGCG on EAE was reported by Aktas *et al*.[3] The authors administered EGCG to female SJL/J mice by gavage (300 μg/mouse twice daily) starting at the day of immunization with PLP$_{139–151}$ significantly reduced symptoms, brain inflammation, and neuronal damage. LN cells isolated from the EGCG-treated mice had lower proliferative response after restimulation with the recall antigen proteolipid protein (PLP)

peptide$_{139–151}$. The authors also showed lower TNF-α production but no effect on IFN-γ and IL-4 production after restimulation with PMA and ionomycin. Although the authors concluded a lack of EGCG effect on Treg cells, this should be interpreted with caution due to the fact that in this study, Treg cells were defined as the phenotype $CD4^+CD25^+$ rather than $CD4^+CD25^+Foxp3^+$, a widely accepted phenotype today for defining Treg cells. Since a majority of activated T cells express CD25, some of $CD4^+CD25^+$ cells may merely represent activated $CD4^+$ T cells rather than Treg cells. Recently, we conducted a multidosing study to determine the preventive and therapeutic efficacy of EGCG in EAE as well as the immunological mechanisms for its action.[6] In this study, female C57BL/6 mice were fed a diet containing 0% (control), 0.15%, 0.3%, or 0.6% (w/w) EGCG for 30 days at which time mice were immunized with MOG$_{35–55}$. The results showed an EGCG dose-dependent attenuation in clinical symptoms and pathological characteristics (inflammation and demyelination). EGCG treatment was found to reduce infiltration of $CD4^+$ T cells, and also of neutrophils and macrophages but to a lesser degree, in the CNS. EGCG consumption was also found to suppress expansion of the autoreactive pathological T cells as well as their proinflammatory effector function after restimulation with the autoantigen MOG$_{35-55}$, as manifested by lower ex vivo (T-cell proliferation) and in vivo (delayed-type hypersensitivity skin test) immune responses. More specifically, compared with the control mice, EGCG-fed mice had smaller populations of Th1 and Th17 cells, and downregulated expression of their corresponding master regulators T-bet and RORγt, respectively, together with a larger population of Treg cells, as measured in LNs, spleen, and CNS. In accordance with this, EGCG-fed mice also had reduced production of several proinflammatory cytokines.

As initial evidence to help us understand inhibitory effect of EGCG on inflammatory infiltration, we found that the EAE mice fed on EGCG had lower levels of soluble intercellular adhesion molecule-1 in plasma and fewer $CD4^+$ T cells expressing C-C chemokine receptor 6 (CCR6) compared with the EAE mice in the control group. Adhesion molecules regulate immune cell migration including the process of moving across the blood–brain barrier. In particular, human studies have shown higher serum levels of soluble ICAM-1 in patients with MS,[67 68] and animal studies also support the importance of ICAM-1 in promoting CNS pathological cell infiltration and disease development in EAE models.[69 70] Regarding the roles of chemokines and their receptors, CCR6 is shown to facilitate entry of the first wave of autoreactive Th17 cells into the uninflamed CNS by interacting with its only ligand CCL20, which is constitutively expressed in the epithelial cells of the choroid plexus.[71] However, CCL20 may not be involved in the EGCG-induced reduction of T-cell infiltration because CCL20 expression in the CNS did not differ between EGCG-fed and control EAE mice.[6] Together these results provide an explanation from the mechanistic aspect for the EGCG-induced reduction in T-cell infiltration into the CNS in EAE mice.

In our study, because mice continued to consume EGCG throughout the experiment including the postimmunization period, it is difficult to prove whether EGCG has a preventive effect on EAE. However, it does appear that EGCG's effect on EAE is more therapeutic rather than preventive based on the observation that feeding EGCG for 30 days prior to immunization delayed onset and reduced severity, but had no effect on the incidence of the disease. Starting EGCG treatment at days 7 and 12 postimmunization also similarly attenuated the symptoms except that EGCG no longer delayed onset when the treatment was started at day 12 postimmunization. These results generated from the EAE model not only validate the clinical relevance of the immunomodulating effect of EGCG but also advance our understanding of the working mechanisms for this beneficial effect of EGCG.

CONCLUDING REMARKS

Current evidence supports the role of green tea EGCG as favorably modulating immune and inflammatory responses, which may be associated with its protective effect in autoimmune disease animal models. Collectively, the experimental observations seem to support the suggested benefits of consuming green tea/EGCG as an alternative and complimentary approach to the goals of developing preventive and therapeutic strategies for autoimmune inflammatory diseases. However, the research in this field is still in its early stages, and the results thus far are promising but not without controversy. It is important to emphasize that convincing evidence for EGCG's effectiveness in human autoimmune disease is largely absent. While animal models are convenient and useful tools for the research in this area, we should recognize their limitations, including the difference between the animal models and the human diseases which these models try to mimic as well as the difference in EGCG's bioavailability and biopotency between animals and humans. Therefore, caution should be taken when interpreting the findings in animal studies and attempting to extrapolate these results to the clinical application. That being said, future intervention studies in humans are needed in order to ultimately determine whether EGCG has potential clinical significance in the amelioration and/or prevention of autoimmune diseases.

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