

Green Tea (*Camelia Sinensis*) Mediated Suppression of IgE Production By Peripheral Blood Mononuclear Cells of Allergic Asthmatic Humans

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Abstract

Recent studies in our laboratory demonstrated the suppression of immunoglobulin E (IgE) production by green tea extract (GTE) in U266 cells. However, the effects of GTE or one of its components (EGCG) on IgE production by human peripheral blood mononuclear cells (PBMC) are unknown. PBMC (1.5×10^6) obtained from serum IgE+, allergic asthmatic patients, were cultured $\pm$ GTE (1–100 ng/ml) or purified EGCG (0.5–50 ng/ml), and IgE levels were determined on day 10 by enzyme-linked immunosorbent assay (ELISA). High levels of IgE were detected in supernatants of the PBMC cultures on day 10. When GTE was included *in vitro*, IgE production by PBMC was suppressed on day 10, compared with control. Purified EGCG included *in vitro* also suppressed IgE production, but at lower levels, compared with control. This study demonstrates that GTE and its major catechin, EGCG, have immunoregulatory effects on human IgE responses.

Introduction

Green tea (*Camelia sinensis*), known for its anti-oxidant, anti-cancer and other beneficial properties [1–7], contains bioactive ingredients, including polyphenols, catechins and caffeine [1, 2]. The catechin epigallocatechin gallate (EGCG) inhibits mast cell degranulation, neutrophil chemotaxis and type IV allergic responses [1, 8]. The *O*-methylated derivative of EGCG, (-)-epigallocatechin-3-*O*-(3-*O*-methyl)-gallate (EGCG''3Me), inhibits type I and IV hypersensitivity reactions [1] and also inhibits histamine release in the human basophilic cell line KU812 [9]. Gallic acid (3, 4, 5-trihydroxybenzoic acid), a green tea polyphenol, modulates the inflammatory allergic reaction by decreasing/blocking IgE-induced histamine release from mast cells, as well as pro-inflammatory cytokine expression [10]. Recent studies in our laboratory have demonstrated the suppression of IgE production by

green tea extract (GTE) in U266 cells, which was not mediated by apoptosis or cell death [11]. However, whether this effect is mediated by EGCG alone or in conjunction with other compounds in GTE has yet to be established.

Human allergic asthma, a disease characterized by airway hyperresponsiveness and bronchial inflammation, is a major health problem that affects approximately 25% of the population worldwide [12]. The innate immune system contributes to airway inflammation in asthma [13] and is mediated by activated leucocytes, including eosinophils [14, 15], mast cells [14, 15], CD4+ T lymphocytes [16] and B cells [17]. It is well established that IgE plays a major role in asthma and allergic reactions through its ability to bind to Fc-epsilon receptor I on mast cells [18].

In this study, we demonstrate that GTE and purified EGCG suppress *in vitro* induction of human IgE

responses in a dose-dependent fashion, suggesting a potential and safe therapeutic option for treating asthma and other diseases of altered IgE regulation.

Methods

Study participants. Peripheral blood (40 ml) was obtained from $n = 3$ allergic asthmatic patients (2 men and 1 woman, age 30–45 years old), from the State University of New York (SUNY) Downstate Asthma Center of Excellence (Table 1). Asthmatic patients presented with clinically defined severe-persistent asthma (e.g. have asthma symptoms throughout the day, use rescue inhaler multiple times a day, have a FEV₁ <60% of predicted) [19], atopy (skin prick positive to at least one of the following panel: Ragweed [Short, Tall], 5 Grass Mix [Timothy, Orchard, June, Red Top, Sweet Vernal], English Plantain, 10 Tree Mix [Ash, Beech, Birch, Elm, Hickory, Maple, Oak, Poplar, Sycamore, Alder]) and perennial allergens Dust Mite (Dermatophagoides pteronyssinus and/or Dermatophagoides farinae, Hollister-Stier, Spokane, WA), American Cockroach, Cladosporium, Alternaria, Dog epithelium, and/or cat pelt (Alk-Abello, Round Rock, TX, USA), and allergic rhinoconjunctivitis, with elevated serum IgE levels (681–2368 IU/ml). None of the subjects received allergen immunotherapy within the prior 6 months. Asthma treatment regimen included as needed inhaled β -agonists and corticosteroids. Written informed consent was obtained from the study participants. The study was approved by the SUNY Downstate Medical Center Institutional Review Board, and the procedures followed were in accordance with institutional guidelines involving human subjects.

Total Serum IgM, IgG, IgA. Blood was collected and immunoglobulin (Ig) levels (IgM, IgG, IgA) were detected in serum. All serum Ig determinations were carried out using nephelometry performed according to manufacturer's recommendations in the Clinical Diagnostic Laboratory at

SUNY Downstate Medical Center (reference range for healthy adult serum: IgM: 47–367 mg/dl; IgG: 648–2045 mg/dl; IgA: 55–375 mg/dl; IgE: 20–100 IU/ml.)

Total Serum IgE. Blood was collected and immunoglobulin E (IgE) levels were determined using the Uni-Cap Total IgE fluoroenzyme immunoassay (Pharmacia and Upjohn Diagnostics) performed according to the manufacturer's recommendations (reference range for healthy adult serum: 20–100 IU/ml). All tests were performed in the Clinical Diagnostic Laboratory at SUNY Downstate Medical Center (Brooklyn, NY, USA).

Fresh green tea extract. Whole green tea (*Camelia sinensis* L) extract (Topix Pharmaceuticals, West Babylon, NY, USA) was suspended in RPMI-1640 (Sigma, St. Louis, MO, USA) at a concentration of 1 g/100 ml and further diluted for the experiments. The extract contained a 90% polyphenol isolate from whole leaf, with 80% catechins; EGCG composed 70% of catechins. GTE was freshly prepared prior to each experiment, and leftover solution was stored at 4°C.

Epigallocatechin Gallate. Purified EGCG (>95% purity; Sigma-Aldrich, St. Louis, MO, USA) was suspended in RPMI-1640 (Sigma) at a concentration of 1 g/100 ml and further diluted to concentrations of 50% because of the 50% content of EGCG in the GTE used.

The GTE contained 90% polyphenols, and 80% of the polyphenols are catechins. 70% of the catechins are EGCG, which approximates to 50% of the GTE is EGCG. Based on the above, the EGCG concentration in culture was 50% of the GTE concentration.

Cell Cultures. Human PBMC (1.5×10^6 cells/ml) were separated on a Ficoll-Paque (Pharmacia, Piscataway, NJ, USA) gradient (density 1.077) and washed twice in RPMI-1640 medium (Gibco/BRL, Grand Island, NY, USA) and counted. Cells were then cultured in complete RPMI medium (c-RPMI) containing L-glutamine (2 mM) (Sigma), penicillin (100 Units/ml) (Sigma), streptomycin (100 μ g/ml) (Sigma) and N-2-hydroxyethylpiperazine-

Table 1 Characteristics of the subjects.

Variable	Subject		
	A	B	C
Age (years)	30	45	31
Sex	F	F	M
Ethnicity	Asian	African American	African American
Serum IgE (IU/ml)	1107	681	2368
Serum IgG (mg/dl)	1482	974	1503
Serum IgM (mg/dl)	141	129	124
Serum IgA (mg/dl)	354	253	157
Skin-prick test positivity	Cat pelt, dog epithelium, cockroach, mouse, ragweed [short, tall], 5 Grass Mix, 10 Tree Mix, English Plantain, alternaria, clad	Cat pelt, American cockroach, English Plantain, dust mite, 5 Grass Mix, alternaria, clad, ragweed [short, tall], dog epithelium, mouse, 10 Tree Mix	Cat pelt, 5 Grass Mix, ragweed [short, tall], dust mite
Atopic disorders	Asthma, rhinoconjunctivitis, eczema	Asthma, rhinoconjunctivitis	Asthma, rhinoconjunctivitis

Reference range for healthy adult serum: IgM: 47–367 mg/dl; IgG: 648–2045 mg/dl; IgA: 55–375 mg/dl; IgE: 20–100 IU/ml.

N'-2-ethanesulfonic acid buffer (HEPES) (25 mM) (Sigma) and supplemented with heat-inactivated foetal calf serum (FCS) (10%) (Gibco), $\pm$ recombinant human interleukin-4 (IL-4) (100 ng/ml) (R&D), $\pm$ mouse anti-human monoclonal (mAb) CD40 (1 μ g/ml) (BD Pharmingen Transduction Labs, San Diego, CA, USA), $\pm$ varying concentrations of GTE (1–100 ng/ml) (Topix Pharmaceuticals, West Babylon, NY, USA) or EGCG (0.5–50 ng/ml) (Sigma). In some experiments, cat pelt antigen (1 AU/ml) (Alk-Abelló, Hørsholm, Denmark) was added to cultures to assess for differences between allergen- and non-specific IgE responses; cat pelt was chosen because all three subjects had positive SPT to cat pelt. Control cultures included anti-CD40 and rhIL-4 without cat pelt antigen. The cells were then cultured for 10 days at 37°C in a humidified atmosphere of 4% CO₂ in air, after which supernatants were collected and frozen (–20°C), and then assayed for IgE production. (ELISA, BioQuant, San Diego CA, USA).

Cell viability. Cell viability was >90% as judged by trypan blue (Gibco) exclusion on day 10 in all cultures ($\pm$ GTE).

Quantification of IgE production. *In vitro* quantitative determination of IgE content in cell culture supernatants was performed using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (IgE ELISA Test Kits, BIOQUANT). All ELISAs were performed according to the manufacturer's recommended procedure. Specimens were analysed in triplicate and a standard curve was derived from known concentrations of IgE. Plates were read using an automated microplate reader (Model Elx800; Bio-Tek Instruments, Winooski, VT, USA), with a 450-nm measurement filter. Optical densities were converted to IU/ml and/or ng/ml based on the standard curve. (1 IU/ml = 2.4 ng/ml).

Statistical analysis. Data are presented as mean $\pm$ standard deviation (SD). Comparisons between variables were performed using general linear models with IgE levels *in*

vitro modelled using repeated measures to control for duplicate experiments and the experimental condition as the independent variable, including age, sex and number of positive SPT as covariates. Given the small sample size, Kruskal–Wallis tests were also performed to confirm significant differences without making any assumptions about the data distribution. The results of the two analyses were similar and general linear models are presented.

A two-tailed *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc, Cary, NC, USA).

Results

Effect of GTE on non-allergen-specific IgE responses

When PBMC from asthmatic patients were cultured for 10 days with anti-CD40 mAb and rhIL-4, high levels of IgE were detected in supernatants on day 10 (8.2 ± 4.7 IU) (Fig. 1A). IgE responses were not detected when PBMC were cultured with either anti-CD40 mAb or rhIL-4 alone (<1.0 IU/ml) (Fig. 1A).

When 1, 10 or 100 ng/ml of GTE was added to cultures, IgE production was suppressed in a dose-dependent manner ($89.3 \pm 5.7\%$, $56.9 \pm 8.9\%$, $0.2 \pm 4.1\%$, respectively), compared with control (general linear models, *P* = 0.07, <0.0001, and <0.0001, respectively) (Fig. 1B).

Effect of purified EGCG on non-allergen-specific IgE responses

When 5 or 50 ng/ml of EGCG was added to cultures, IgE production was also suppressed in a dose-dependent manner ($87.0 \pm 7.0\%$ and $72.6 \pm 14.4\%$, respectively), compared with none (*P* = 0.02 and <0.0001, respectively) (Fig. 1C). However, 0.5 ng/ml of EGCG did not significantly suppress the IgE production ($95.7 \pm 3.8\%$, *P* = 0.90).

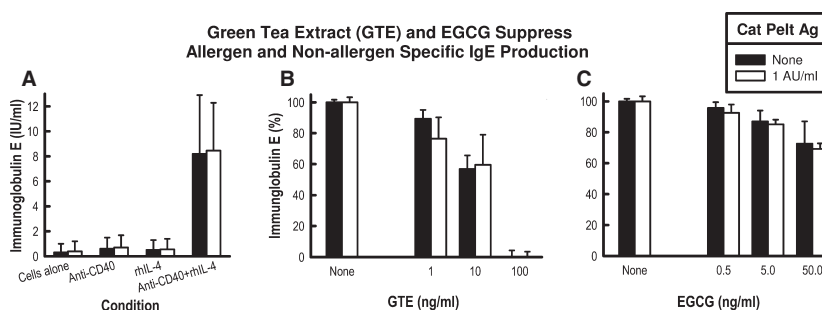


Figure 1 Green tea extract (GTE) and EGCG suppress allergen and non-allergen-specific IgE production. PBMC (1.5×10^6) from allergic subjects (*N* = 3) were cultured in c-RPMI (see Methods), $\pm$ cat pelt antigen (1 AU/ml), $\pm$ varying concentrations of GTE (1–100 ng) (Panel B) or $\pm$ varying concentrations of EGCG (0.5–50 ng) (Panel C) or without GTE or EGCG $\pm$ anti-CD40 mAb or $\pm$ rh IL-4 or $\pm$ anti-CD40 + rhIL-4 (Panel A) for 10 days, after which levels of IgE in supernatants were determined (ELISA). Data are expressed as either IU/ml $\pm$ standard deviation (SD) or mean IgE expressed as % control (untreated) IgE $\pm$ SD.

Effect of GTE on allergen-specific IgE responses (cat pelt)

When PBMC from asthmatic patients were cultured for 10 days with the addition of cat pelt antigen (1 AU/ml), high levels of IgE were also detected in supernatants on day 10 (8.5 ± 3.8 IU) (Fig. 1A).

When 1, 10 or 100 ng/ml of GTE was added to cultures, IgE production was suppressed in a dose-dependent manner ($76.4 \pm 13.8\%$, $59.5 \pm 19.5\%$, $0.2 \pm 3.3\%$, respectively), compared with control (general linear models, $P = 0.001$, <0.0001 , <0.0001 , respectively) (Fig. 1B).

Effect of purified EGCG on allergen-specific IgE responses (cat pelt)

When 50 ng/ml of EGCG was added to culture, IgE production was also suppressed in a dose-dependent manner ($69.2 \pm 3.7\%$), compared with control ($P = 0.002$ and <0.0001 , respectively) (Fig. 1C). However, 0.5 and 5 ng/ml of EGCG did not significantly suppress IgE production ($94.1 \pm 4.8\%$ and $85.0 \pm 3.1\%$, $P = 0.73$ and 0.06 , respectively).

Discussion

This study demonstrates that GTE or its catechin EGCG suppresses *in vitro* allergen- and non-allergen-specific IgE production in human PBMC from allergic asthmatics (up to 98%). Our findings suggest that GTE or EGCG has immunoregulatory effects on human IgE responses. Treatment of individuals with asthma and allergies may benefit from safe and alternative therapeutic options such as natural plant derivatives, whose clinical importance may be underestimated.

Previous studies in our laboratory established that GTE suppresses B cell production of IgE without inducing apoptosis, in a homogeneous U266 B-cell model [11]. In this study, cell viability in PBMC was observed ($>90\%$) on day 10 in the presence or absence of GTE to rule out cytotoxicity as a potential mechanism of GTE's inhibitory potential on IgE production and is in agreement with our earlier studies that GTE suppresses *in vitro* IgE responses without inducing apoptosis [11].

It has been demonstrated that green tea polyphenols, including epicatechin-3-gallate (ECG) and EGCG, exhibit anti-mutagenic and anti-carcinogenic activity in microbial systems, mammalian cell systems and *in vivo* [20]. Studies of Nakazato, *et al.* [21] reported that ECG has potential as a novel therapeutic agent for patients with B-cell malignancies (e.g. multiple myeloma), possibly through induction of apoptosis mediated by modification of the redox system [21]. GTE has been shown to inhibit breast cancer growth by a direct anti-proliferative effect on the tumour cells as well as by indirect suppressive effects on the tumour-associated

endothelial cells [22] and can increase the inhibitory effect of tamoxifen on the proliferation of the oestrogen receptor MCF-7, ZR75, T47D human breast cancer cells *in vitro* [22]. Studies of Silverberg *et al.* [23] found that GTE inhibits hydrogen peroxide-induced necrosis of human skin fibroblasts [23]. In various tumour cell systems, green tea polyphenols have been implicated in induction of apoptosis, via a caspase 3-executed mechanism that targeted the mitochondria [24].

In other disease states, GTE also prevented Abeta [25]-induced activation of NF- κ B, ERK and P38 MAP kinase pathways in rats, suggesting that GTE may prevent the development and progression of Alzheimer's disease [25]. Green tea extract-4 (CSI-4) has also been reported to possess anti-adhesive activity against certain pathogenic bacteria (e.g. *P. acnes*), with no adverse effects against beneficial bacteria (e.g. *Lactobacillus acidophilus*) [26]. Previous studies of Nie *et al.* [27] demonstrated that green tea polyphenols and their major component, EGCG at a concentration of 200 microM, exert significant protective effects against 6-OHDA-induced PC12 cell apoptosis, and EGCG was more effective than the mixture of green tea polyphenols [27]. The authors concluded that green tea polyphenols' neuroprotective effect was because of antioxidant function [27] and has potential for the treatment of neurodegenerative diseases [27].

In this study, addition of GTE (1–100 ng/ml) resulted in suppression of IgE (up to 98%); EGCG (0.5–50 ng/ml) alone moderately suppressed IgE production (up to 28%). Addition of cat pelt antigen (1 AU/ml) and GTE (1–100 ng/ml) or EGCG (0.5–50 ng/ml) also resulted in suppression of IgE production (up to 31% and 98%, respectively). However, addition of 0.5 ng EGCG did not suppress IgE production. Some of the active components in GTE, other than EGCG, might have contributed either additively or synergistically to the total IgE suppression observed. We used unseparated GTE because this likely closely mimics the advantageous effects of green tea, in that it includes all of the potentially bioactive ingredients a human-consuming green tea would receive. The GTE contained 90% polyphenols, and 80% of the polyphenols are catechins. 70% of the catechins are EGCG, which approximates to 50% of the GTE is EGCG. Based on the above, the EGCG concentration in culture was 50% of the GTE concentration.

Published studies investigating the effect of GTE on development of allergic disease are inconclusive, with some reporting deleterious effects and increased risk for inducing asthma [28–30]. However, in those studies, green tea-induced asthma was reported in individuals who worked in green tea factories. It may be that excess occupational exposure to green tea results in a hyperresponsiveness to green tea or its components, which would not be applicable to the general population. Future studies, including mechanism, are warranted to determine whether

individual catechins (e.g. EGCG) or other plant extracts result in suppression of IgE production *in vivo*.

This study has potential limitations including small study/sample size; future studies will be performed on a larger scale to increase our sample size. In addition, PBMC from non-allergic/non-asthmatic healthy controls do not produce IgE responses *in vitro* [39]. Thus, this group was not studied. However, the strengths of this study are (1) that our results are highly relevant to addressing potential safe treatments for allergic asthma and possible other atopic conditions and (2) that these *in vitro* studies can be the framework for further exploration of this topic both *in vitro* and *in vivo*.

In summary, this study demonstrates GTE and EGCG suppression of human IgE production *in vitro*. These results may lead to future improvements in asthma treatment and prevention.

Disclosure

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