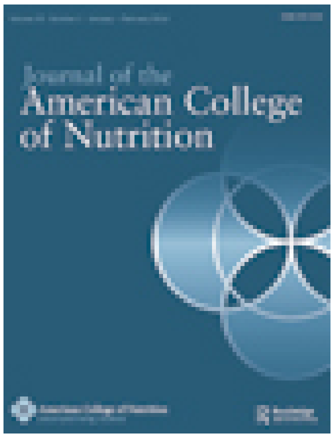

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Specific Formulation of Camellia sinensis Prevents Cold and Flu Symptoms and Enhances γδ T Cell Function: A Randomized, Double-Blind, Placebo-Controlled Study

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Objective: Determine if a specific formulation of *Camellia sinensis* (CSF) can prevent illness and symptoms due to cold and flu, and enhance γδ T cell function

Methods: Design: Randomized, double-blind, placebo-controlled study. Subjects: Healthy adults 18–70 years old. Intervention: Proprietary formulation of *Camellia sinensis* (green tea) capsules, or a placebo, twice a day, for 3 months. Measures of Outcome: As assessed by daily symptom logs, percentage of subjects experiencing cold and flu symptoms, number of days subjects experienced symptoms, and percentage of subjects seeking medical treatment. Mean *in vivo* and *ex vivo* proliferative and interferon gamma responses of subjects' peripheral blood mononuclear cells to γδ T cell antigen stimulation.

Results: Among subjects taking CSF there were 32.1% fewer subjects with symptoms ($P = 0.035$), 22.9% fewer overall illnesses of at least 2 days duration ($P = 0.092$), and 35.6% fewer symptom days ($P < 0.002$), compared to subjects taking placebo. γδ T cells from subjects taking CSF proliferated 28% more ($P = 0.017$) and secreted 26% more IFN-γ ($P = 0.046$) in response to γδ T cell antigens, as compared to γδ T cells from subjects taking placebo. CSF was well-tolerated.

Conclusions: This proprietary formulation of CSF is a safe and effective dietary supplement for preventing cold and flu symptoms, and for enhancing γδ T cell function.

INTRODUCTION

For centuries, tea beverage has been linked to good health. Most studies have been observational, showing benefit in cardiovascular, anti-aging, neurodegenerative, anti-cancer, and bone areas [1,2]. There are numerous other studies consistent with no health benefits [3,4]. These studies, both positive and negative, are fraught with confounding variables that are inherent in observational studies employing free-living human beings as subjects. Such studies are further complicated by the nature of tea beverage. There are hundreds of varieties of the tea species, *Camellia sinensis,* and numerous ways to process tea that can lead to different components in a cup of tea [5,6]. Storing and brewing methods lead to further variability that is difficult to standardize. Another obvious source of conflict is that there is no general agreement on what quantity constitutes a cup of tea. Thus, it is not surprising that the results of many published trials conflict with one another.

Whereas most clinical tea research has focused on cardiac and cancer outcomes, there are no available clinical human data on prevention of cold and flu symptoms. These symptoms are the most common afflictions in humans, resulting in misery, loss of productivity, and absence from work and school [6]. Further, recent studies that call in to question the efficacy of zinc-based nasal sprays and lozenges [7], and Echinacea [8] make new research into cold and flu more important. There is emerging evidence that components found in tea beverage may have anti-infective and anti-inflammatory effects.

L-theanine, an amino acid found uniquely in tea, is catabolized to ethylamine, a molecule that specifically activates

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Abbreviations: CSF, *Camellia sinensis* formulation; EGCG, epigallocatechin gallate, PBMC, peripheral blood mononuclear cells; IFN-γ, interferon gamma.

Contribution by JFB was in his capacity as NSRI Director; he is also a consultant for Taiyo International.

Dr. Cheryl A. Rowe and Meri P. Nantz are equal contributors to the work.

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human γδ T lymphocytes to proliferate and make interferon gamma, a powerful antimicrobial cytokine [9,10]. Subjects who drank six cups of tea per day had up to a 15-fold increase in interferon gamma production in as little as one week, when their peripheral blood mononuclear cells (PBMC) were exposed to bacteria [10]. Coffee drinking had no such effect.

There is ample evidence supporting the role of γδ T cells as a first line defense against infection. In mice, studies using specific γδ T cell mAbs and γδ T cell knockout mice provide compelling evidence for protective roles against bacterial, parasitic, and viral infection [11–15]. In humans, γδ T cells expand up to 50-fold in the peripheral blood 6- to 10 days after infection with many bacterial, viral, and parasitic microbes [16–20]. These γδ T cell expansions can be reproduced *in vitro* by exposure of PBMC to purified nonpeptide microbial products (alkylamines and organophosphates), pharmacologic agents that mimic bacterial antigens, such as nitrogen-containing bisphosphonates, as well as heat killed bacteria and parasites, bacterial or parasitic extracts, and virus-infected cells [21–24]. Using a severe combined immunodeficiency mouse reconstituted with human PBMC (hu-SCID) model, Wang et al. showed that the presence of human γδ T cells is necessary for survival during infection by *E. coli* or *M. morganii,* and that protection is associated with secretion of large quantities of gamma interferon (IFN-γ) by these γδ T cells [25]. The antibacterial effect of human γδ T cells *in vivo* is evident as early as 17h post-infection, indicating that γδ T cell expansion is not required for an antibacterial response and suggesting that γδ T cell-mediated cytokine secretion is a crucial component of protection. PBMC primed with alkylamine or nitrogen-containing bisphosphonate antigens such as risedronate and pamidronate, specific for Vγ2Vδ2 T cells, produced higher amounts of IFN-γ and provided more protection against infection than unprimed PBMC [25].

Epigallocatechin gallate (EGCG), a powerful catechin antioxidant found in green and white teas, can directly kill bacteria and viruses, including the influenza virus [26–29]. EGCG is also highly anti-inflammatory. This activity is potentially important in cold and flu infections, since soluble mediators of inflammation cause symptoms. EGCG inhibits the production of pro-inflammatory mediators such as chemokines [30,31], prostaglandins [31], and tumor necrosis factor (TNF) [32]. EGCG also inhibits adhesion molecule expression [33], MAP kinases [34], and neutrophil migration [35].

Based on these promising data, we hypothesized that ingestion of tea extracts containing standardized amounts of the tea components L-theanine and EGCG would enhance systemic immunity, and prevent cold and flu symptoms in healthy individuals. We conducted a randomized, double-blind, placebo-controlled interventional study using a specific proprietary formulation of these key tea components (*Camellia sinensis* formulation; CSF) with a primary endpoint of reduction in the number of subjects who developed illness due to cold and flu. We describe here results showing that this CSF reduces by about one third the number of subjects who develop illness due to cold and flu, while enhancing γδ T cell proliferation and IFN-γ secretion in response to antigen.

MATERIALS AND METHODS

Subjects

Healthy men (n = 52) and women (n = 72) between 21 and 70 (mean = 29) years of age were recruited to participate in a 12-wk randomized, double-blind placebo controlled parallel study. Subjects were recruited from the University of Florida campus, and the Gainesville, Florida community, during January of 2006. The University of Florida Institutional Review Board approved the study protocol, and informed written consent was obtained from each subject. Screening for the study occurred by telephone and/or personal interviews. Exclusion criteria consisted of the following: had not had a cold in the past two years; vegetarian diet; steroids; chemotherapy or other immune suppressing therapy within the last year; chronic antibiotics or other infectious disease preventative; chronic illness; recent surgery or illness; pregnant and/or lactating females. Also excluded were those who daily consumed: greater than one cup (250mL) of tea; an average of seven or more servings of fruits and vegetables; herbal supplements, vitamins other than a multivitamin or vitamin D, or osteoporosis medicine or medications containing bisphosphonates, that are activators of γδ T cells [36]. The study was conducted from late January through early May of 2006. Participants were in contact with the enrolling research assistant by e-mail and telephone throughout the study, and returned to fill out an exit questionnaire upon study completion. Overall study compliance was monitored through the exit questionnaire and by enumeration of remaining capsules in returned bottles at the end of then study [37].

Study Protocol

The study was conducted from late January through early May of 2006, to coincide with normal cold and flu season. The CDC weekly report of influenza activity in Florida was as follows: January-March regional to widespread, March-April widespread to local activity, April-May local activity to sporadic.

Subjects were randomly assigned to supplement and placebo groups. Both subjects and investigators were blinded as to the treatments. Nutraceutical Holdings, LLC (Orlando, FL) provided ImmuneGuard®, a proprietary *Camellia sinensis* formulation (CSF) and placebo capsules. This decaffeinated formulation contains a proprietary mixture of L-theanine (Suntheanine®, standardized at 99%; Taiyo International, Minneapolis, MN), and epigallocatechin gallate (EGCG; Sunphenon®, Taiyo International, Minneapolis, MN, standardized at

50%). The placebo capsules contained microcrystalline cellulose, dextrose, dicalcium phosphate, magnesium stearate, silicon dioxide, FD&C red #40, yellow #6, and blue #1. Each participant was given a bottle containing 180 capsules and was instructed to take 2 capsules every day (one in the morning and one in the evening, preferably with meals) for 12 weeks.

Subjects were given an illness log to record any cold and flu symptoms during the 12 week experimental period. Primary outcomes were defined as the number of subjects experiencing any symptoms, and the mean number of symptom days. For example, if a subject had a cough and a sore throat on the same day, this was counted as two symptom days. For analysis of illness frequency, illness was defined as having one or more symptoms for at least two consecutive days. The symptoms assessed were: runny nose, congested or stuffy nose, headache, cough, sore throat, fever, nausea/vomiting, and diarrhea. Subjects were also asked to report if they sought medical treatment and were prescribed any medications as a result of seeking treatment. The exit questionnaire included questions to determine if subjects experienced any side effects and/or experienced any changes in feelings of stress or anxiety, or took any additional dietary supplements during the study. Finally, subjects were asked to report whether they thought they had taken the active or the placebo capsules.

Blood Collection

Blood was obtained from fasting subjects on Days 0 (baseline), and 21. Blood was collected into one 10mL sodium heparin tubes for peripheral blood mononuclear cell (PBMC) separation, and one 10mL SST™ tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for serum. Tubes for PBMC were maintained at room temperature (RT), while tubes for serum were kept at 4°C. All tubes were processed within 1 hr of blood collection. Blood cell separation and culture procedures were carried out under sterile conditions.

Serum Collection and Treatment

Serum was removed from SST™ tubes after centrifugation (1000g, 10min, 4°C) and frozen at −80°C.

Blood Cell Separation

Whole blood was diluted and placed on a gradient to separate PBMC. Briefly, 7mL of whole, anti-coagulated blood (RT) was diluted 1:1 with 0.9% NaCl. Diluted blood (6mL) was layered over 3mL of Nycoprep 1-Step™ 1.077 (Axis-Shield, Oslo, Norway) and centrifuged (800g, 20 min, 20°C). The mononuclear cell layer was removed, washed twice with 10% FBS-RPMI 1640 (Cellgro; Mediatech, Herndon, VA) complete (100U/mL Penicillin; 100μg/mL Streptomycin; 0.25μg/mL Fungizone; 50μg/mL Gentamycin; 2mM 1-glutamine; 25mM HEPES) by centrifugation (400g, 10min, 4°C). Individual cell pellets were resuspended in 2mL RPMI 1640 complete, without serum, and counted on a Z-2 Coulter Counter (3.8 μ setting for the 100μm aperture tube, Beckman Coulter, Inc., Fullerton, CA).

Culture of Peripheral Blood Mononuclear Cells (PBMC) for $\gamma\delta$ T-Cell Expansion and Cytokine Production

On Day 0, 1.0 × 10^6 PBMC in 10% FBS-RPMI 1640 complete medium containing 50μM 2-ME, were seeded into each of two wells of duplicate 24-well tissue culture plates (Costar, Corning, NY). Ethylamine (1mM/mL, Sigma) in the same medium was added to one set of wells on each plate, while the other set was mock treated with 1mL medium. The plate was incubated in a humidified 5% CO_2 atmosphere at 37°C. On Day 1, cells and supernatant fluids from one plate were harvested and centrifuged (1500, 10min, 4°C). The supernatant fluids were removed and frozen at −80°C for cytokine analysis, while the cell pellets were re-suspended in 0.1mL PBS and 0.5mL RNAlater™ (Ambion, Austin, TX) and frozen at −80°C. On Day 3, 30U/mL of recombinant human IL-2 (BD Biosciences, San Diego, CA) were added to all wells of the remaining plate, which was incubated until Day 10 when cells were harvested. Total cell counts were obtained on a Coulter Counter (4.5μ setting) and dead cells enumerated via trypan blue exclusion on a hemocytometer, and cells processed for flow cytometry.

Flow Cytometry

The ratio of $\alpha\beta$ and $\gamma\delta$ T cells in PBMC suspensions and cultures was determined by flow cytometry, using cell surface markers for identification on Days 0 and 10 of culture.

Antibodies. Phycoerythrin (PE)-conjugated anti-human $CD3^+$ (pan-T cell marker), and fluorescein isothiocyanate (FITC)-conjugated anti-human T cell surface markers $\alpha\beta$TCR (pan-$\alpha\beta$ T cell marker) and $\gamma\delta$TCR (pan-$\gamma\delta$ T cell marker; eBioscience, San Diego, CA) were used to stain PBMC.

Staining/Fixation. PBMC in staining buffer (PBS + 0.1% NaN_3 + 2% FBS) were stained on ice in the dark for 30min, with PE anti-human $CD3^+$ antibody and one of the two FITC-conjugated TCR specific antibodies. Cells were washed (PBS + 0.1% NaN_3) by centrifugation (1000g, 10 min, 20°C). Cells were fixed with 1% paraformaldehyde in PBS + 0.1% NaN_3. Cells were analyzed within 24 hours on a FACScan (Becton-Dickinson, San Jose, CA). Data was analyzed using WinMDI Software (Scripps Institute, build 1301-19-2000). Data collected was from gated $CD3^+$ cells (events). Final data is reported as the mean percentage of cells expressing the specific cell surface marker, ± the standard deviation (SD).

Cytokine Level Determination in Supernatants from Cell Proliferation

Levels of human IFN-γ from PBMC culture supernatants were quantified using enzyme linked-immunosorbent assay

(ELISA) kits, according to the manufacturer's directions (BD Biosciences Pharmingen, San Diego, CA). Human cytokine standards provided with each kit were used for a standard curve. Supernatants were analyzed undiluted for IFN-γ. Plates were read at 450nm (with λ correction of 570nm) on a SPECTRAmax 340PC plate reader (Molecular Devices, Menlo Park, CA). Cytokine concentrations were calculated as the mean value obtained for values within the range of the standard curve. The limits of detection of the cytokine ELISA kits were: IFN-γ -300pg/mL.

Statistical Analysis

For analysis of illness and symptom frequency, the z-test for two proportions, with two-tailed probability was used. Two-way ANOVA, with 2-tailed probability, was used for γδ T cell proliferation studies and IFN-γ secretion.

RESULTS

Study Subjects

One hundred and twenty-four subjects were enrolled in the study. Six subjects withdrew from the study (three *Camellia sinensis* formulation; CSF, and three placebo). Adverse events were mild, infrequent, and transient. Bloating, gastric upset, dizziness, skin rash, and constipation were reported, and were not different between experimental and control groups. Two subjects taking CSF withdrew with mild skin rashes. One subject said she thought the rash was related to a seafood allergy, and the other thought it might be from the capsule. Neither subject sought medical attention. Such food allergies for green tea have been described previously, mostly in tea factory workers, and the causative agent in green tea is EGCG [38]. However, other reports in the literature describe EGCG as beneficial for asthma and atopic dermatitis [39,40].

One placebo subject withdrew due to an unrelated urinary tract infection. One placebo individual withdrew because traveling interfered. One placebo individual withdrew because he could not return. One placebo subject withdrew because he just stopped taking the capsules (Table 1). Five more subjects (two CSF and three placebo) were excluded from all data because they were less than 70% compliant. One more CSF subject was excluded when they reported in their logs that they were ill when the study began, which was an exclusion criterion. Two more CSF subjects and one more placebo subject entered incomplete log data, and were excluded from the analysis. Fifty-five subjects in the supplement group and 53 subjects in the placebo group completed the study with 70% or greater compliance, and their data were included in all clinical results and analyses. Three week blood samples were obtained from 52 CSF and 56 placebo subjects who were complaint over that time period, and were included in the immune function analysis.

Demographics

Average age, gender and BMI values did not differ between experimental and control groups. Subjects were adequately blinded, as there was no difference between CSF and placebo in the percentage of subjects who guessed which treatment to which they were assigned (Table 2).

Ingestion of *Camellia sinensis* Formulation (CSF) Reduced the Number of Subjects Having Cold and Flu Symptoms

Throughout the three month study, subjects were asked to keep an illness log to report daily cold and flu symptoms, including fever, runny nose, stuffy nose, sore throat, cough, fever, headache, diarrhea, and nausea. Thirty-five of 55 (63.6%) placebo subjects, but only 23 of 53 (43.2%) CSF subjects had at least one symptom during the 12 week study. Thus, 32.1% fewer subjects taking CSF experienced cold and flu symptoms as compared to subjects taking placebo ($P = 0.035$; Fig. 1). Three of 53 (5.7%) CSF subjects, and seven of 55 (12.7%) placebo subjects sought medical treatment (58.3% difference; $P = 0.213$).

We analyzed on a monthly basis the number of illnesses and symptom days in each group, from February to May. The study extended only a few days into May, so April and May were combined. There were no significant differences in number of illnesses from month to month. However, there were significantly more symptom days in February (48% of total symptoms, $P < 0.002$ compared to March or April/May; Table 3).

Comparing CSF and placebo subjects, there were no significant differences in the number of illnesses within any single

Table 1. Study Withdrawals

Subject #	Treatment	Capsules Consumed For	Reason for Withdrawal
2	supplement	11 d	Facial & chest rash, itching, red/watery eyes, puffy eyes, congestion)
30	placebo	?	Unable to return
43	supplement	On & off 20 dy (?)	Hives 3×
45	placebo	32 dy	Traveling interfered
60	placebo	1 mo (?)	Urinary tract infection
116	supplement	?	Discontinued taking capsules
119	placebo	1 mo (?)	Capsules upsetting stomach

Table 2. Demographics of the Study Population

	Supplement	Placebo	P value
Age	28.9 ± 1.07	30.3 ± 1.5	0.65
Gender male	20	23	
Gender female	32	33	
Height (m)	1.7 ± 0.01	1.7 ± 0.01	0.60
Weight (kg)	74.9 ± 1.7	73.3 ± 2.0	0.67
BMI	25.4 ± 0.6	24.3 ± 0.8	0.94
Compliance (%)	93% ± 7	93% ± 7	0.56
Blinding (% guessed correctly)	50	48	1.0

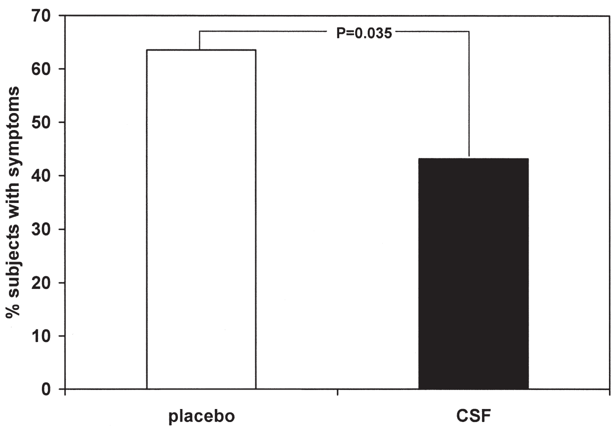

Fig. 1. The percentage of 55 CSF subjects and 53 placebo subjects who had at least one symptom during the 12-week study period.

time period. However, there were 22.9% fewer overall illnesses (P = 0.092) in the CSF group. Only 34 and 36% of symptom days occurred in CSF subjects in February and March, respectively (P < 0.002 compared to placebo). However, 51% of symptom days in April/May occurred in the CSF group, roughly equaling the 49% of symptom days in the placebo group. Overall, 53 CSF subjects had a total of 360 symptom days (mean, 6.8 days), and 55 placebo subjects had a total of 559 symptom days (mean, 10.2 days). This represents a 33.3% reduction in the number of symptom days for CSF subjects as compared to the placebo group (P < 0.022; Table 3).

Taken together, these results show that CSF is effective in preventing cold and flu symptoms in this group of healthy volunteers. The data suggest that CSF may also decrease the duration of symptoms and the need for medical treatment.

Ingestion of *Camellia sinensis* Formulation Enhanced Proliferation and IFN-γ Secretion by $\gamma\delta$ T Cells

Our previous study showed that PBMC taken from subjects who started drinking daily five to six cups of tea, containing L-theanine, for as little as one week secreted significantly more IFN-γ in response to $\gamma\delta$ T cell antigens, as compared to before [10]. To determine if ingestion of CSF, containing L-theanine, could enhance IFN-γ production, we isolated PBMC taken from subjects three weeks after they started taking CSF or placebo, and cultured them for 24 hours with media alone, or with media containing ethylamine, which is a $\gamma\delta$ T cell antigen that is a byproduct of L-theanine catabolism. It is important to note that only $\gamma\delta$ T cells respond to ethylamine [9,41], and that CSF ingestion had no effect on IFN-γ secretion or T cell proliferation in response to a non-specific T cell mitogen, phytohemagglutinin (data not shown). PBMC from CSF and placebo subjects secreted only 2.8 and 2.3 ng/mL IFN-γ, respectively. As expected, PBMC from placebo subjects secreted more IFN-γ in response to 5 mM ethylamine as compared to media alone (13.6 ng/mL). However PBMC from CSF subjects secreted 18.4 ng/mL IFN-γ in response to ethylamine. This response was 26% higher than that from placebo PMBC (P = 0.046; Table 4).

We established separate 10-day PBMC cultures from the subjects to assess by flow cytometry the proliferative responses of $\alpha\beta$ and $\gamma\delta$ T cells. Absolute numbers of $\alpha\beta$ T cells did not change when cultured in media alone, or with ethylamine (data not shown). In the presence of ethylamine, $\gamma\delta$ T cells from CSF subjects expanded to 28% of CD3+ cells, as compared to 20.3% from placebo subjects (P = 0.017; Table 5). Thus, CSF consumption was associated with a significant increase in the capacity of $\gamma\delta$ T cells to secrete IFN-γ and to proliferate in response to antigenic challenge.

DISCUSSION

Fewer than 5% of Americans eat the nine servings per day of fruits and vegetables suggested by the latest USDA guidelines released in 2005 [42]. Only 20% of Americans drink any tea at all, while those who do only drink an average of one to two cups per day. Unfortunately, most health benefits from tea are associated with higher amounts of daily consumption [1]. Tea is a vegetable infusion, containing antioxidants and other beneficial nutrients such as L-theanine. Numerous observational studies suggest that tea drinking is beneficial to health, but negative studies have introduced controversy surrounding its health benefits. Though any two studies can yield different

Table 3. Monthly Illnesses and Symptoms for Subjects Taking CSF and Placebo

	CSF Number of illnesses (n = 53)	Placebo Number of illnesses (n = 55)	z-test p values*	CSF Number of symptom days reported	Placebo Number of symptom days reported	z-test p values*	Percent of total symptom days in CSF
February	15	18	0.618	152**	294**	<0.002	34
March	12	19	0.172	81	142	<0.002	36
April/May	10	11	0.882	127	123	0.720	51
Total	37	48	0.092	360	559	<0.002	39

* Comparison of number of illness in CSF and placebo group, month by month, and total.

^ Comparison of number of symptom days reported in CSF and placebo group, month by month, and total.

** 48% of all symptom days occurred in February ($P < 0.002$ compared to March or April/May).

Table 4. Interferon-γ (ng/mL) Secreted into the Culture Medium after 24h

	Tea formula (n = 52)	Placebo (n = 56)	p values
0 mM ethylamine	2.8^{C}	2.3^{C}	Ethylamine level = 0.001
1 mM ethylamine	3.8^{C}	2.1^{C}	Treatment = 0.046
5 mM ethylamine	18.4^{A}	13.6^{B}	Interaction = 0.084
Pooled SEM	3.9		

Repeated measures two-way ANOVA. Means with different superscripts are statistically different using a post hoc SNK test.

Table 5. % γδ-T Cells in Baseline Blood Sample and after 10d of Culture

	Tea Formula (n = 52)	Placebo (n = 56)	p values
Baseline (no culture)	13.2 ± 4.6	14.3 ± 6.6	0.270
0 ethylamine, 10 d	20.8 ± 7.0	21.9 ± 5.7	0.298
1 mM ethylamine, 10 d	28.0 ± 5.0	20.3 ± 4.0	0.017

Repeated measures two-way ANOVA; means ± SEM.

results, the conflict between negative and positive studies is likely due in large measure to the observational nature of the studies, and differences in tea preparations (see introduction). We have conducted a randomized, double blind placebo-controlled study using a proprietary *Camellia sinensis* formulation (CSF) with defined amounts of L-theanine and EGCG that as closely as possible approximates the ingestion of 10 cups of green tea per day. The strength of this design lies in elimination of subject selection bias inherent in observational studies. Another strength is the elimination of the variability that can be associated with tea varieties and tea beverage preparations.

The results show that the ingestion of two capsules daily decreased by about a third the number of subjects who experienced cold and flu symptoms. Also seen were decreases in the number of days subjects had symptoms, and the number of subjects needing medical treatment. The magnitude of reduction of these symptoms in a preventive manner has enormous implications for public health.

There was a significantly increased cluster of symptom days, and a suggestion of an increased cluster of illness in February as compared to March and April/May. This is consistent with a colder climate in northern Florida during February, and this decreased temperature might be expected to be associated with more cold and flu activity. There was an unexpected decrease in the efficacy of CSF during April/May. This decrease may be due to waning compliance with a twice daily regimen at the end of the trial, or waning efficacy of the CSF. While overall compliance was measured by pill counts, we did not measure compliance month by month, so this end of trial decrease in compliance remains speculative. Further studies will be necessary to examine the cause of this decreased CSF effect.

The CSF benefits seen were not related to sex, gender, race, or BMI. CSF was well-tolerated. There were no significant differences in adverse events, or withdrawls due to adverse events.

In addition to its effect on the incidence of cold and flu symptoms, CSF ingestion enhanced proliferation of, and IFN-γ secretion by, γδ T cells challenged *in vitro* with ethylamine. These findings are consistent with our previous study showing that subjects who drank tea containing L-theanine, but not coffee, increased their γδ T cell function by up to 15-fold [10]. It is important to point out that γδ T cell numbers or serum IFN-γ levels were not increased in subjects taking CSF, but that their γδ T cells were primed *in vivo* by CSF to respond more vigorously to antigenic challenge *ex vivo*. Constitutive increases in γδ T cell numbers and serum IFN-γ titers in the absence of pathogen exposure may actually increase symptoms due to inflammation. Such increased symptoms occur after ingestion of high doses of nitrogen-containing bisphosphonates (which activate γδ T cells) for treatment of osteoporosis [43].

The mechanism of action of symptom reduction has not been determined with certainty. However, γδ T lymphocytes are an important first line of defense against microbes [25]. L-theanine is known to prime human γδ T lymphocytes to secrete IFN-γ, a powerful antimicrobial cytokine [9,10]. In the early stages of infection, EGCG may aid in the activation of γδ T cells by enhancing secretion of IL-12, a very important factor in γδ T cell activation [10,44]. EGCG also kills viruses and bacteria directly [26–29], and can decrease virus titers *in vivo* during chronic viral infection [45]. If infection does establish itself by evading the first-line γδ T cell response, EGCG may decrease cold and flu symptoms due its anti-inflammatory activity [35]. Studies in our laboratory are now underway to address these mechanistic questions.

This study has several limitations. Symptom data were collected by self-reporting, so an actual medical diagnosis of cold or flu was not possible. Other illnesses, such as pneumonia, bronchitis, or allergy might have caused similar symptoms. The study was comprised of only healthy adult subjects, so it was not possible to assess the effect of CSF on children, or subjects with chronic illnesses who have increased susceptibility to acute illness.

Cold and flu symptoms can be perennial sources of misery and lost productivity for most healthy adults, and the introduction of a safe, effective, and natural capsule that can prevent such symptoms represents a significant breakthrough in preventive medicine.

CONCLUSION

This proprietary *Camellia sinensis* formulation (CSF) is an effective preventative for cold and flu symptoms, and for enhancing the innate immune response. Widespread use of this CSF could have enormous beneficial effects in decreasing morbidity in healthy populations.

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