Gamma tocopherol-enriched supplement reduces sputum eosinophilia and endotoxin-induced sputum neutrophilia in volunteers with asthma

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Background: We and others have shown that the gamma tocopherol (γT) isoform of vitamin E has multiple anti-inflammatory and antioxidant actions and that γT supplementation reduces eosinophilic and endotoxin (LPS)-induced neutrophilic airway inflammation in animal models and healthy human volunteers.
Objective: We sought to determine whether γT supplementation reduces eosinophilic airway inflammation and acute neutrophilic response to inhaled LPS challenge in volunteers with asthma.
Methods: Participants with mild asthma were enrolled in a double-blinded, placebo-controlled crossover study to assess the effect of 1200 mg of γT daily for 14 days on sputum eosinophils, mucins, and cytokines. We also assessed the effect on acute inflammatory response to inhaled LPS challenge following γT treatment, focusing on changes in sputum neutrophilia, mucins, and cytokines. Mucociliary clearance was measured using gamma scintigraphy.
Results: Fifteen subjects with mild asthma completed both arms of the study. Compared with placebo, γT notably reduced pre-LPS challenge sputum eosinophils and mucins, including mucin 5AC and reduced LPS-induced airway neutrophil recruitment 6 and 24 hours after challenge. Mucociliary clearance was slowed 4 hours postchallenge in the placebo group but not in the γT treatment group. Total sputum mucins (but not mucin 5AC) were reduced at 24 hours postchallenge during γT treatment compared with placebo.
Conclusions: When compared with placebo, γT supplementation for 14 days reduced inflammatory features of asthma, including sputum eosinophils and mucins, as well as acute airway response to inhaled LPS challenge. Larger scale clinical trials are needed to assess the efficacy of γT supplements as a complementary or steroid-sparing treatment for asthma. (J Allergy Clin Immunol 2018;141:1231-8.)

Key words: *Asthma, endotoxin, eosinophil, lipopolysaccharide, mucin, mucociliary clearance, neutrophil, tocopherol, vitamin E*

Asthma is among the most prevalent chronic diseases in the United States[1] and represents a source of significant burden to patients and health care systems. Environmental pollutant exposure is a known trigger for asthma exacerbations, which are characterized by airway inflammation, bronchoconstriction, increased production of airway mucous, and decreased mucociliary clearance (MCC) with formation of mucous plugs.[2,3] Endotoxin (the main component of which is LPS) is commonly encountered in ambient air particulate matter as well as in domestic and occupational settings and has been linked to asthma severity.[4-6] Endotoxin is a potent stimulator of the innate immune response,[7] signaling through Toll-like receptor 4 on airway macrophages to stimulate production of inflammatory cytokines and eicosanoids, recruitment of granulocytes, and production of gel-forming airway mucins, including mucin 5AC (MUC5AC).[8]

Airway inflammation during acute exacerbations of asthma is often characterized by increase in both airway eosinophils and neutrophils.[9] Neutrophilic airway inflammation is particularly evident in viral asthma exacerbations as well as in some chronic asthma phenotypes.[10] Our group has shown that inhaled LPS challenge induces airway neutrophilia in human volunteers, and we now employ this procedure as a model of acute exacerbation of airway disease against which potential therapies can be tested.[11-13] We have previously demonstrated that inhaled fluticasone propionate administered for 2 weeks decreased

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The Effect of Gamma Tocopherol Enriched Supplementation on Response to Inhaled LPS (Vitalps) study is registered at ClinicalTrials.gov as NCT02104505.

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Abbreviations used

αT: α-Tocopherol
γ-CEHC: 2,7,8-Trimethyl-2-(β-carboxyethyl)-6-hydroxychroman
γT: γ-Tocopherol
MCC: Mucociliary clearance
MUC5AC: Mucin 5AC
%PMNs: Sputum percentage of neutrophils

sputum eosinophilia and subsequent LPS-induced acute airway neutrophilia in asthmatics.[14] In subsequent studies, we have shown that treatment with the IL-1 receptor antagonist, anakinra,[15] and the vitamin E isoform, gamma tocopherol (γT)[16] also attenuated LPS-induced airway neutrophilia in healthy volunteers.

Our hypothesis that vitamin E supplementation decreases airway inflammation in asthma and allergic airway disease was inspired by epidemiologic evidence suggesting that increased dietary vitamin E intake is associated with reduced incidence of allergic disease[17-19] and asthma.[20] Among the isoforms of vitamin E that have been suggested as asthma interventions are α-tocopherol (αT), which is commonly used as both a supplement and pharmaceutical product, and γT, the predominant isoform of vitamin E found in dietary sources. Intervention trials of αT in humans with asthma have been generally disappointing.[21,22]

γT has not been as vigorously studied for airway disease. γT and its primary metabolite 2,7,8-trimethyl-2-(B-carboxy-ethyl)-6-hydroxychroman (γ-CEHC) do have a number of unique anti-inflammatory actions,[23,24] including scavenging reactive nitrogen species to form 5-nitro-γ-tocopherol[24] and inhibition of COX-2 and 5-lipooxygenase, reducing inflammatory eicosanoid production.[25] We have pursued a program of preclinical and early phase clinical trials of γT as a novel therapeutic for treatment of airway inflammation.[25-29] In a rodent model of evoked airway inflammation, γT reduced allergen-induced eosinophilia and mucin responses[27] as well as LPS-induced neutrophil, prostaglandin E2, and mucin responses (including MUC5AC).[29] We subsequently observed that 1 week of treatment with a γT-enriched mixed tocopherol preparation reduced the neutrophilic response to inhaled LPS challenge in a phase I randomized, double-blinded, placebo-controlled crossover study of healthy adults.[16] This report describes our next step in assessing γT as an intervention for asthma, in which we test the hypothesis that γT reduces eosinophilic airway inflammation and attenuates the neutrophilic airway response to inhaled LPS challenge in volunteers with mild asthma.

METHODS

Volunteer recruitment and inclusion criteria

We recruited subjects aged 18 to 50 years with a history of episodic wheezing or shortness of breath consistent with asthma or physician-diagnosed asthma classified as mild intermittent or mild persistent asthma as defined by the National Heart, Lung, and Blood Institute guidelines for the Diagnosis and Management of Asthma.[30] Exclusion criteria included any of the following: daily albuterol use, nighttime asthma symptoms more than once per week, or emergency treatment for asthma within the previous 12 months. As sputum inflammatory cell measures were a central endpoint in this study, all subjects were screened for their ability to provide an adequate induced sputum sample during their screening session, defined by >250,000 cells, >50% viability, and <40% squamous cells.

Prior to study entry, subjects underwent a general health screen including a detailed medical history, physical exam, baseline laboratory evaluation, spirometry, and allergy skin testing to common aeroallergens including house dust mite, cockroach, tree mix, grass mix, weed mix, molds, cat, dog, guinea pig, rabbit, rat, and mouse allergens. A wheal size of 3 mm or greater than the negative control was considered positive. Subjects who were found to be pregnant, nursing an infant, regularly taking anti-inflammatory or immune-modulating medications, or with a history of abnormal blood coagulation parameters were excluded. This study was approved by the University of North Carolina Institutional Review Board and the US Food and Drug Administration (IND 13004) and is listed on ClinicalTrials.gov (NCT02104505).

Study design

Subjects were randomized to 1200 mg γT or placebo (safflower oil) treatment for 14 days (Fig 1), a study period similar to that used to assess the effect of fluticasone propionate on airway response to LPS challenge in asthmatics. The γT supplement consisted of gel tabs each containing 612 mg γT, 7 mg αT, 28 mg β-tocopherol, and 8 mg δ-tocopherol (Callion Pharma, Inc, Jonesborough, Tenn), and subjects were instructed to take 2 gel tabs once daily with a meal to maximize bile secretion and enhance absorption. Medication bottles were returned and any leftover pills were counted on the day of LPS challenge to ensure adherence. Twenty-four to 48 hours prior to LPS challenge, subjects presented for sputum induction and gamma scintigraphy to measure MCC. On day 14 of dosing, subjects underwent inhaled LPS challenge with 20,000 endotoxin units of Clinical Center Reference Endotoxin, with MCC measurement performed 4 hours postchallenge and sputum induction at 6 and 24 hours postchallenge. Sputum was analyzed for granulocytes, inflammatory cytokines, and mucin content as previously described.[31-34] After a minimum 3-week washout to allow for clearance of inflammatory cells from the airways, subjects were crossed over to the alternate treatment group. Venipuncture was performed at regular intervals for assessment of prothrombin time, activated partial thromboplastin time, and international normalized ratio.

Randomization and masking

The randomization list was prepared by a biostatistician using SAS 9.4 (SAS Institute, Cary, NC) and provided to the University of North Carolina Investigational Drug Service. Only the pharmacist and statistician had access to the randomization schedule. Subjects were randomized to treatment groups 1:1 using permuted blocks of 4. γT and safflower oil (placebo) gel tabs were identical in appearance and were dispensed as a 7-day supply from the Investigational Drug Service to the study staff. Subjects returned for a follow-up visit to receive the additional 7-day supply of investigational drug for that period.

Endotoxin inhalation challenge

Clinical Center Reference Endotoxin, referred to as LPS, was provided by the National Institutes of Health Clinical Center. All doses were prepared by the Investigational Drug Service and inhaled by subjects as a nebulized preparation using a DeVilbiss Ultraneb 99 ultrasonic nebulizer (DeVilbiss, Port Washington, NY).[12,16]

Sputum induction, processing, and mediator measurement

Each subject provided 7 induced sputum samples (Fig 1): screening (prior to placebo or active treatment), 24 to 48 hours prior to each LPS challenge session (posttreatment sputum), and 6 and 24 hours after each LPS challenge session (postchallenge sputum). Induced sputum samples were processed as previously described.[31-34] Cell viability (trypan blue exclusion) and total cell counts were assessed in a Neubauer hemacytometer (Fisher Scientific, Hampton, NH), and differential cell counts were performed on cytocentrifuged cells stained with a modified Wright stain (Hema-Stain-3; Fisher Scientific, Hampton, NH). Cytokines from sputum supernatants were measured using multiplex technology (Meso Scale Discovery/MSD, Gaithersburg, Md). Each sample was analyzed

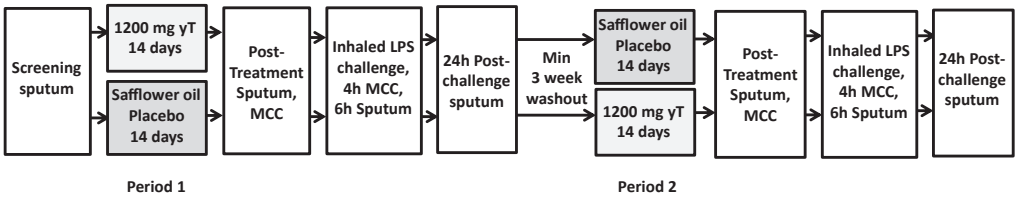

FIG 1. Phase IIa crossover study design in volunteers with mild asthma (n = 15). Randomized, placebo controlled crossover study of γT supplement or safflower oil placebo in 15 subjects with mild asthma. Subjects were challenged with inhaled LPS followed 4 hours later by gamma scintigraphy to measure MCC and 6 hours later by sputum induction.

with the V-PLEX Human Proinflammatory Panel II kit (Meso Scale). Even though ability to provide adequate sputum for analysis was an entrance criterion, there were instances during the study in which a subject was not able to provide a sputum sample or provided a poor-quality sputum sample. In these instances, these volunteers were excluded from analysis of the effect of active treatment on airway inflammation. These volunteers were included in assessment of MCC and safety end points for the study.

Gamma scintigraphy for measurement of MCC

The procedure used for measuring MCC in humans has been described in detail previously.[35,36] Briefly, volunteers inhaled an aerosol of technetium Tc 99msulfur colloid using a slow inhalation (80 mL/sec), large particle (9.5 μm mass median aerodynamic diameter) method.[37] Immediately following inhalation of the radioaerosol (duration of <5 minutes), an initial deposition scan was recorded (sum of two 2-minute images) and then continuous 2-minute images were recorded for a period of 2 hours to monitor clearance of particles from the lung as the subject remained seated in front of the gamma camera. Subjects returned the following day after the radiolabeled aerosol exposure to obtain a 30-minute scan of 24-hour lung activity/retention.

A whole lung region of interest bordering the right lung (created from a Co57 transmission lung scan[37]) was used to determine, by computer analysis, the whole lung retention (decay and background corrected) as a fraction of the initial counts in the right lung, over the 2-hour clearance period at 10-minute intervals. MCC was calculated and expressed as average clearance in percent over the 2-hour period.[35] Because measures of MCC can be influenced by the initial, regional lung deposition of the inhaled radioaerosol, we also calculated (1) the central/peripheral ratio of activity and (2) the skew of the counts/pixel versus number of pixels histogram for the initial 2-minute image from each study visit.[35,38]

Analysis of serum tocopherols and γ-CEHC

αT, γT, δ-tocopherol, and 5-nitro-γ-tocopherol were measured by an HPLC assay with electrochemical detection,[39] and γ-CEHC was analyzed using liquid chromatography tandem mass spectrometry as previously described.[40]

Analysis of sputum mucins

To measure total mucins, a 100 μL aliquot of induced sputum was solubilized in 6MGuHCl and subjected to differential refractometry (tREX, Wyatt Technology, Goleta, Calif) coupled with size exclusion chromatography as described previously.[41] Individual concentration of MUC5AC was measured by labeled mass spectrometry method using deuterium-labeled MUC5AC peptide standards.

Statistical analysis

We employed methods similar to those used in our initial study of the effect of γT in healthy volunteers.[16] The primary end points of the study were airway eosinophilia (defined as the difference in sputum eosinophils present in posttreatment samples) and LPS-induced airway neutrophilia (defined as the change in induced sputum neutrophils (PMNs) from posttreatment to 6 hours postchallenge), comparing γT treatment with placebo.

In planning this study, we were guided by the results of our previous study of γT-enriched supplementation on airway PMN response to LPS challenge in 13 healthy volunteers.[16] Based on these data, we estimated that a sample size of 30 volunteers would be adequate for this study, with an *a priori* plan to undertake an interim analysis after 15 volunteers completed this study. As planned and approved by Institutional Review Board and US Food and Drug Administration review, the interim analysis would lead us to stop the study due to demonstration of futility or statistically significant support of the hypotheses that γT inhibits airway eosinophilia and LPS-induced neutrophilia, or continuation of the study to n = 30 due to inconclusive interim results.

For initial posttreatment versus postchallenge comparisons of sputum end points and MCC within each treatment group, paired *t* tests or Wilcoxon signed rank tests (depending on whether the normality assumption was met) were employed. Data that were not normally distributed were transformed using Box-Cox transformation. Given the crossover design of our study, we next determined the γT treatment effect (compared with placebo) on posttreatment sputum end points and on LPS-induced changes (Δ postchallenge – posttreatment) in sputum end points using a linear mixed model approach described by Jones and Kenward[42] that considers the above-mentioned individual tests in a global, unified way where all data are used at the same time. SAS PROC MIXED was used to fit the linear mixed model. Criterion for significance was taken to be $P \leq .05$.

RESULTS

Subject demographics

Twenty-three subjects with mild asthma were enrolled and underwent randomization. Based on frequency of daytime and nighttime symptoms and use of rescue albuterol, 22 subjects were classified as having mild intermittent asthma. One subject was classified as having mild persistent asthma and was using montelukast daily at the time of enrollment. However, this subject withdrew from the study prior to inhaled LPS exposure. No subjects were using inhaled corticosteroids at the time of enrollment or at any point during the study. The majority of participants were atopic (74%) based on the results of skin prick testing. Demographic characteristics of the study population are shown in Table I. Fifteen subjects completed both arms of the crossover study (see Fig E1 in the article's Online Repository at www.jacionline.org), with 13 providing adequate sputum for assessment of the primary sputum inflammatory end points for both treatment periods.

γT supplementation increased serum γT and γ-CEHC concentrations

Serum γT and γ-CEHC concentrations rose significantly from baseline values in the active treatment group only ($P < .0001$ for both) (Table II). Conversely, αT

TABLE I. Demographic characteristics of enrolled study volunteers (N = 23)

Age (y), median (range)	26 (20-47)
Sex (female/male)	19/4
Race	
Caucasian	15
African American	4
Asian	2
Native American	2
Ethnicity	
Hispanic	1
Non-Hispanic	22
Atopic, n (%)	17 (74)
BMI (kg/m^2), median (range)	26 (20-42)
FEV_1 (L), median (range)	3.3 (2.4-4.4)
FEV1 % predicted, median (range)	97 (83-109)

BMI, Body mass index.

concentrations decreased from baseline in the active treatment group ($P = .003$).

γT treatment reduced posttreatment sputum eosinophils and mucins

Using the linear mixed model approach, we found that γT treatment significantly reduced posttreatment sputum percentage of eosinophils ($P = .04$) and eosinophils per milligram of sputum ($P = .01$) compared with placebo (Fig 2, *A* and *B*). Likewise, γT treatment significantly reduced posttreatment total mucins ($P = .03$) and MUC5AC content ($P < .0001$) compared with placebo (Fig 2, *C* and *D*).

γT treatment attenuated LPS-induced sputum neutrophilia

Inhaled LPS challenge significantly increased sputum percentage of neutrophils (%PMNs) ($P = .003$) and neutrophils per milligram (PMNs/mg) of sputum ($P = .01$) at 6 hours compared with posttreatment sputum during the placebo period. The increase during the placebo period (%PMNs: Δ 20.1 ± 16.5%, $P < .01$; PMNs/mg Δ 384.1 ± 531.2, $P < .01$) was greater than that seen during the active period (%PMNs: Δ 11.7 ± 20.7%, $P = .04$; PMNs/mg: Δ 236.2 ± 692.7, $P = .2$). Linear mixed modeling demonstrated that γT treatment (compared with placebo) significantly attenuated sputum %PMNs at both 6 ($P = .04$) and 24 hours ($P = .02$) after LPS challenge (Fig 3, *A* and *B*). There was no effect of inhaled LPS challenge or γT treatment on any measure of sputum eosinophilia following LPS challenge.

γT effects on airway mucin production and MCC

MUC5AC content was significantly increased from posttreatment levels in both treatment groups 6 hours after inhaled LPS challenge ($P = .001$ [placebo], $P = .0004$ [active]). By 24 hours post-LPS challenge, total sputum mucins decreased in both treatment groups compared with prior to LPS challenge, though not significantly. Using linear mixed modeling to assess for a treatment affect, we detected significantly less total sputum mucins during the active treatment period compared with the total associated with placebo use ($P = .03$) (Fig 3, *C*). We found no significant difference in MUC5AC concentrations between the treatment groups at the same time point (data not shown).

As an exploratory measure, we assessed how γT intervention may impact MCC. MCC was measured prior to and 4 hours after LPS challenge. There were no differences in regional deposition indices (central/peripheral ratio or skew) nor in 24-hour retention between posttreatment and postchallenge measurements for either treatment period. MCC was significantly slowed following LPS challenge compared with posttreatment measurements for the placebo treatment period (MCC = 16.3 ± 9.3% postchallenge vs 21.4 ± 6.9% posttreatment, $P < .01$) (Fig 4). In contrast, there was no such slowing of MCC by LPS challenge during active treatment (MCC = 20.2 ± 8.0% postchallenge vs 21.4 ± 9.7% posttreatment, $P = .6$). However, for this new end point, the sample size was not adequate to definitively ascribe a treatment effect for γT on LPS-induced slowing of MCC when accounting for period and carryover effects.

γT treatment did not impact LPS-induced changes in sputum T_H1 cytokines

During the placebo period, sputum IL-1β and IL-8 concentrations were significantly increased 6 hours post-LPS challenge compared with posttreatment values ($P = .002$ and $P = .01$, respectively), while no significant LPS-induced increase was observed during the active treatment period ($P = .07$ and $P = .40$, respectively). There was no significant LPS-induced change in sputum IL-6 concentration during either treatment period. Compared with placebo treatment, we did not detect a significant γT treatment effect on LPS-induced inflammatory cytokine concentrations in sputum following LPS challenge.

Adverse events

No serious adverse events occurred during the study period. The most commonly reported symptoms were gastrointestinal in nature. During the active treatment period, 21.7% of subjects reported nausea and 26% reported diarrhea or loose stools, compared with 8.7% and 4.3% during the placebo treatment period, respectively. These symptoms were typically transient and tended to occur during the first or second day of treatment and then self-resolved. One subject chose to discontinue study participation due to intolerable diarrhea during the active treatment period. No significant change in prothrombin time, activated partial thromboplastin time, or international normalized ratio was observed, and there were no reported bleeding events during the study. After completion of 14 days of active treatment, no clinically or statistically significant changes were seen in FEV_1 or FEV_1/forced vital capacity from measurements taken during the initial baseline visit.

DISCUSSION

The primary goal of this proof-of-concept study was to determine whether γT supplementation in adults with asthma decreases airway eosinophilia as well as the inflammatory response to inhaled LPS, a model of neutrophil-predominant asthma exacerbation. Our results demonstrate that asthmatics treated with γT supplementation for 14 days had significantly reduced eosinophils in sputum when compared with those receiving placebo treatment. These findings suggest that γT may reduce baseline T_H2-mediated airway inflammation, which could be beneficial for eosinophilic asthma phenotypes. We also

TABLE II. Serum concentrations of tocopherols and γ-CEHC from 18 volunteers with mild asthma

	Baseline	Placebo treatment period	Active treatment period
γT (μmol/L)	2.6 (1.43-6.22)	3.69 (1.65-8.82)	19.8 (2.49-50.15)*
αT (μmol/L)	25.41 (14.72-37.81)	25.22 (18.48-52.65)	19.04 (11.07-36.52)*
δT (μmol/L)	0.09 (0.01-0.68)	0.12 (0.04-0.4)	0.26 (0.06-0.6)
γ-CEHC (μmol/L)	0.14 (0.05-0.45)	0.17 (0.07-1.4)	3.11 (0.08-7.82)*
5-NO_2-γT (μmol/L)	0.01 (0-0.07)	0.02 (0-0.07)	0.01 (0-0.05)

Data represented as medians (ranges).
5-NO_2-γT, 5-nitro-γ-tocopherol; *δT*, δ tocopherol.
*$P < .05$ comparing baseline concentrations to those obtained after 14 days of γT supplementation. Analyses were performed using paired *t* tests for γT and αT data and by Wilcoxon matched pairs signed rank test for δT and γ-CEHC data as they were not normally distributed.

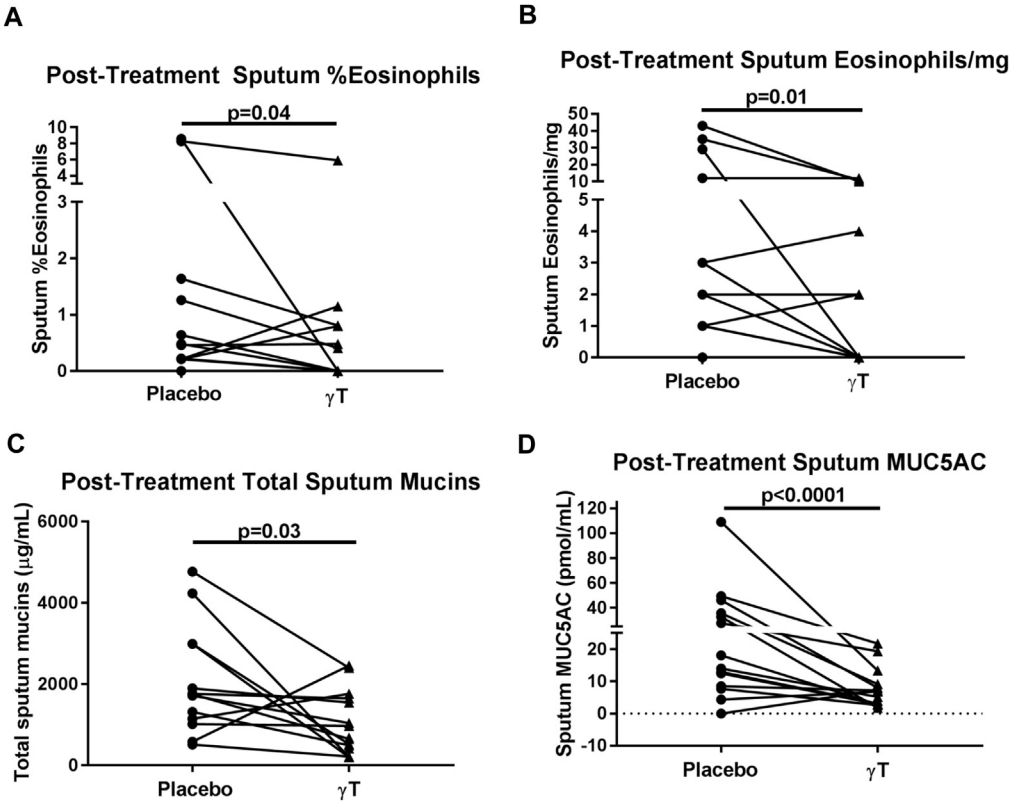

FIG 2. γT reduced posttreatment sputum eosinophils and mucins (n = 13). Sputum %eosinophils **(A)**, sputum eosinophils/mg **(B)**, total sputum mucins **(C)**, and sputum MUC5AC concentrations **(D)** were reduced in posttreatment sputum samples during active treatment compared with placebo treatment.

found that, compared with placebo, γT treatment was associated with lower posttreatment sputum mucins, including the inducible mucin glycoprotein, MUC5AC, which has been found in high concentrations in mucous plugs from fatal asthma cases.[43] *Ex vivo* γT treatment was previously found to inhibit IL-13-induced secretion of eotaxin from airway epithelial cells, a potent chemotactic factor for eosinophils.[44] Given that mucin production is enhanced by IL-13, similar mechanisms may account for the impact of γT on mucin production as well.

We also found that γT treatment attenuated neutrophilic airway response to inhaled LPS challenge. Neutrophilic airway inflammation is often less responsive to corticosteroid treatment,[45] and there is a great unmet need for nonsteroidal therapies that target this specific type of inflammation. While we found no significant difference in sputum mucins between the treatment periods at 6 hours post-LPS challenge, total mucins were significantly lower at 24 hours with γT treatment, which could suggest faster recovery from mucin hypersecretion following an acute inflammatory challenge.

We observed a significant impairment of MCC following inhaled LPS challenge during the placebo treatment period but not during the γT treatment period. We have previously found a slowing of MCC by LPS challenge in healthy nonsmokers[35] and a trend toward slowing in mild asthmatics that was confounded by regional deposition differences between baseline and post-LPS challenge MCC (unreported).[38] While this study was not powered to detect a significant treatment effect of γT on MCC, our results do suggest that γT reduces LPS-induced slowing of MCC, and warrants further study. The mechanism by which inhaled LPS slows MCC is not understood, but it may be related to quantity

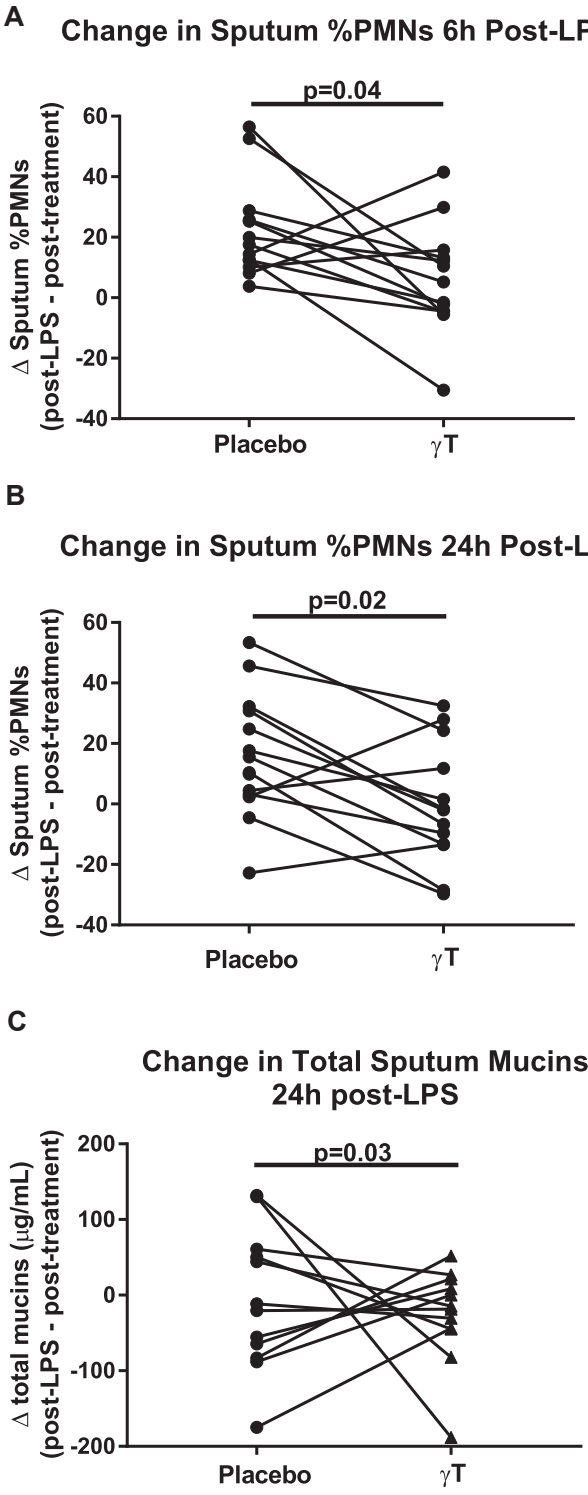

FIG 3. γT attenuated LPS-induced sputum neutrophilia and mucin production (n = 13). Sputum %PMNs at 6 hours **(A)** and 24 hours **(B)** postchallenge were significantly reduced during active treatment compared with placebo. **C,** Total sputum mucins at 24 hours postchallenge were significantly reduced during active treatment compared with placebo treatment.

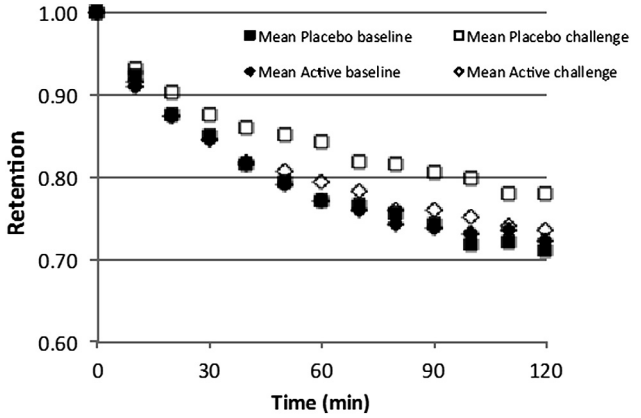

FIG 4. γT was associated with attenuation of LPS-induced changes in MCC (n = 15). MCC is represented as mean retention versus time at posttreatment and 4 hours postchallenge for each treatment group. Inhaled LPS challenge resulted in significant slowing of MCC after placebo treatment, but no significant effect on MCC was seen after γT treatment.

or quality of sputum mucins, epithelial tethering of mucins, direct effects on ciliary function, or a combination of these factors.[43]

The γT supplement used in our study given daily over a 2-week period resulted in significantly increased serum concentrations of γT and its primary active metabolite γ-CEHC but with reduced serum αT concentrations by an unknown mechanism. This finding is consistent with previous studies of γT supplementation effects on reducing αT plasma concentrations,[46] including one conducted by our group that demonstrated reduced αT concentrations in serum following a 3-dose regimen of an identical γT supplement administered over a 24-hour period.[47] It is unknown whether continued γT supplementation would result in further decline in αT concentrations, nor is it known what the long-term physiologic consequences of this decline would be.

While our work has consistently demonstrated a beneficial effect of γT on airway inflammation, others have proposed a proinflammatory role for γT based primarily on human observational or animal model studies. In a cross-sectional study of young adults enrolled in the Coronary Artery Risk Development in Young Adults (CARDIA) cohort, higher serum αT levels were associated with higher lung function values (FEV_1 and forced vital capacity), while higher serum γT levels were associated with lower FEV_1 and forced vital capacity values.[48] While the results of this epidemiological study are intriguing, the correlation between serum γT levels and lung function may reflect γT as a risk factor or biological marker for lung function. Furthermore, others have shown that dietary vitamin E, at least 70% of which is composed of γT, was associated with increased FEV_1 in older adults[49] and may be protective against adult-onset asthma.[20] It is important to emphasize that these studies were not intervention trials, and several potential confounding factors could have influenced their results, including differences in intake of dietary fats. For example, γT-rich oils tend to have higher levels of polyunsaturated fatty acids, which may contribute to certain disease states, whereas αT-rich oils contain predominantly monounsaturated fatty acids, which have more health benefits.[50] Although further studies are needed to address the long-term impact of γT supplementation on airway inflammation, our 2-week dosing regimen with γT had no impact on spirometry measurements.

There are very few published human trials of γT supplementation in the context of airway inflammation prevention and/or treatment. Vitamin E has been studied for prevention and treatment of many chronic health conditions,[51-54]

yet human trials in asthma have yielded conflicting results and have focused on treatment with αT, the most abundant tocopherol isoform in widely available supplements. In contrast to the results presented here, studies utilizing murine models found that γT supplementation exacerbated eosinophilic inflammation, while αT supplementation conferred protection.[55,56] It is possible that these conflicting reports reflect species-dependent differences in the anti-inflammatory effects of γT. Previous work from our group demonstrates that γT supplementation reduces airway eosinophilia in humans[16] and rodents.[27,28] This is in agreement with our current study, in which short-term dosing with γT exhibits acute anti-eosinophilic and anti-inflammatory properties in human subjects. These results, coupled with evidence that γT has unique anti-inflammatory properties compared with αT (including the ability to scavenge reactive nitrogen species[24] and inhibit COX-2 and 5-lipooxygenase[25]) supports the use of γT-enriched vitamin E preparations as a potential intervention for acute exacerbation, pollution induced disease, and possibly even chronic allergic diseases. These findings support conducting larger trials with γT supplementation in volunteers with asthma to further evaluate its role in modulating features of asthma.

This early phase clinical trial has several limitations. Our participants were predominantly female, which could reduce the generalizability of our results. The study population was somewhat heterogeneous with both atopic (74%) and nonatopic (26%) participants, and based on our safety criteria to undergo inhaled LPS challenges, had mild asthma. Given that the supplementation period only lasted 2 weeks, the longer-term effect of reduced serum αT levels noted with γT supplementation will have to be further studied for safety and efficacy in treating chronic airway inflammation. Our dosing regimen was generally well-tolerated, though early, transient gastrointestinal symptoms occurred in about one-fourth of participants studied. Additionally, we saw no prolongation of blood coagulation measurements, and no significant bleeding events were reported. The occurrence of early gastrointestinal side effects and potential need for both long-term and short-term treatment regimens suggests that dose ranging studies need to be done. Finally, the impact of body mass index on driving treatment responses to LPS will have to be further evaluated, given that we have previously shown that increased BMI is associated with sputum neutrophil recruitment to inhaled LPS among atopic subjects with asthma.[57]

In conclusion, we have shown that a 14-day course of γT supplementation resulted in reduced eosinophilic inflammation of the airways and reduction in sputum mucins including MUC5AC. Additionally, γT supplementation reduced LPS-induced neutrophilic airway inflammation and mucinous content of sputum following inhalation challenge and was associated with reduced impact of LPS on MCC. Overall, our results with 2 weeks of γT supplementation were similar to the effects of 2 weeks of treatment with inhaled fluticasone propionate on both posttreatment sputum eosinophilia and acute neutrophilic response to inhaled LPS challenge.[14] Taken together, these observations indicate that γT has potential to treat multiple features of asthma, including airway inflammation, mucous production, and clearance of mucous from the airways, and should be studied further in larger-scale clinical trials to investigate the efficacy of γT for improving asthma outcomes.

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Key messages

- **γT supplementation in mild asthmatics reduced sputum eosinophils and mucins compared with placebo in a fashion similar to that of inhaled fluticasone propionate and may have a role in reducing T_H2-mediated inflammation.**
- **γT reduced the neutrophilic inflammatory response to inhaled LPS challenge compared with placebo and may represent a useful therapy for neutrophil-predominant asthma exacerbation.**

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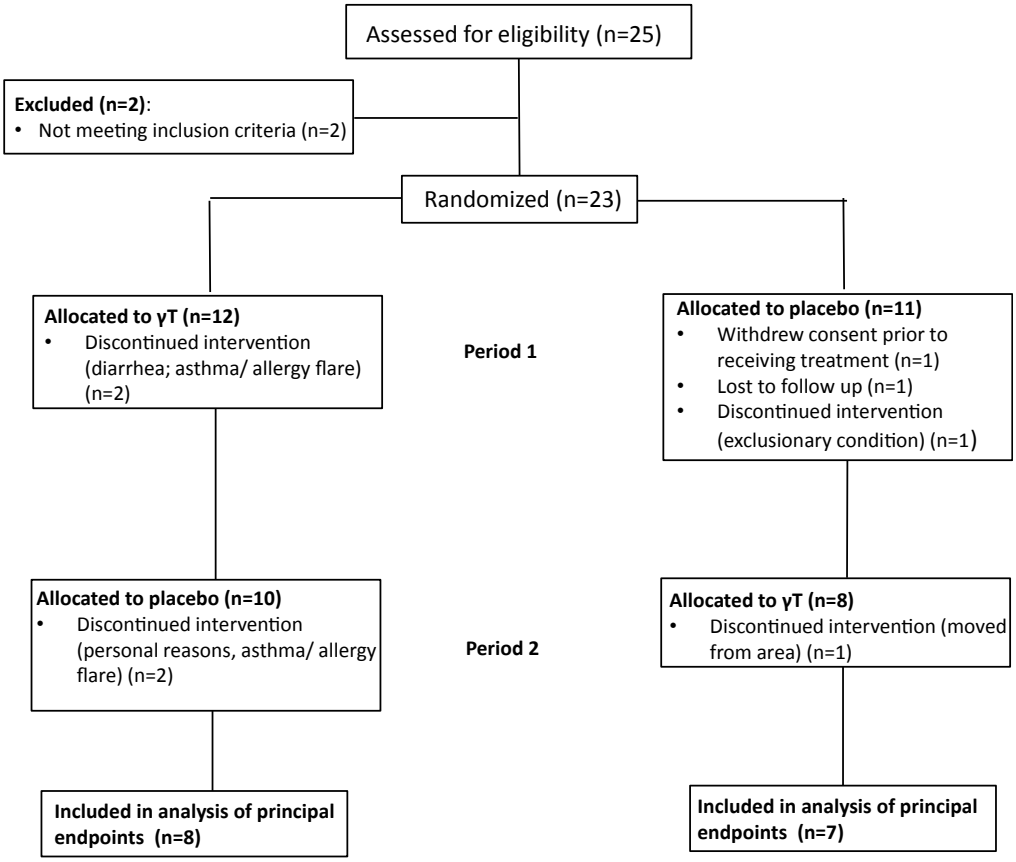

FIG E1. CONSORT diagram outlining subject randomization and participation in a phase IIa crossover study of volunteers with mild asthma.