

BRIEF COMMUNICATION

Vitamin E, γ -tocopherol, diminishes *ex vivo* basophil response to dust mite allergen

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Abstract

Epidemiologic studies suggest that dietary vitamin E is a candidate intervention for atopic disease. We used *in vitro* and *ex vivo* exposures to test the hypothesis that the most common dietary isoform of vitamin E, γ -tocopherol (γ T), could suppress Fc ϵ RI-mediated basophil activation. Rat basophilic leukemia (RBL)-SX38 cells that express human Fc ϵ RI were treated with or without γ T, followed by stimulation with α -IgE. In the *ex vivo* study, 20 *Der f 1*-allergic volunteers consumed a γ T-enriched supplement for 7 days. Their basophils were challenged *ex vivo* with α -IgE and graded doses of *Der f 1* before and after the supplementation period. γ T treatment of RBL-SX38 cells significantly reduced basophil degranulation and de novo TH2 cytokine production. Daily consumption of a γ T-rich supplement by dust mite-allergic volunteers reduced basophil activation after *ex vivo* dust mite challenge. Vitamin E supplements rich in γ T may be useful adjuncts in decreasing atopic disease.

Complementary and alternative medicine therapies have become increasingly popular in the United States and Europe for the treatment of allergies and asthma (1, 2). Vitamin E is a nutritional antioxidant, which has been studied for its impact in asthma. Decreased levels of IgE and reduced occurrence of childhood asthma have been associated with intake of dietary vitamin E (predominantly gamma tocopherol, γ T) early in life (3) or in children of women with high vitamin E intakes during pregnancy (4). In a rodent model of allergic rhinitis and asthma, we found that daily administration of γ T prior to Ova challenge depressed eosinophil infiltration into the airways, as well as production of nasal TH2 cytokines and pulmonary eicosanoids (5).

Previous work showed that vitamin E interfered with phosphoinositide-3-kinase and protein kinase C activation (6),

required for IgE-mediated degranulation and cytokine production by mast cells and basophils. As an initial step in exploring the utility of γ T as an adjunctive treatment for atopy, we assessed the effect of γ T *in vitro* on IgE-mediated degranulation and mediator production using a rat basophilic leukemia cell line, RBL SX-38, which stably expresses human Fc ϵ RI $\alpha\beta\gamma 2$. Based on promising *in vitro* findings, we then examined the role of consuming a γ T-enriched preparation on IgE-mediated basophil activation in a proof-of-concept *ex vivo* study in dust mite-allergic volunteers.

RBL SX-38 cells were kindly provided by Jean-Pierre Kinet from the Beth Israel Deaconess Medical Center (7). The release assay was adapted from Blanc et al. (8). Cells were sensitized with 0.5 μ g/ml human IgE (Abbiotec, San Diego, CA, USA) or fresh media (MEM with 10% FBS from Hyclone) for 24 h. The next day, 20 μ M γ T, vehicle (0.1% DMSO), or 1 μ M cyclosporine was added and incubated for 16 h overnight. The following morning, cells were washed with NaPIPES buffer followed by stimulation with 10 μ g/ml anti-human IgE antibody (α -IgE, Bethyl Labs, Montgomery, TX, USA) for 60 min (7). All conditions were run in

Abbreviations

CysLTs, cysteinyl leukotrienes; *Der f 1*, *Dermatophagoides farinae* 1 antigen; IL-13, Interleukin-13; IL-4, Interleukin-4; RBL, rat basophilic leukemia; rIL-3, recombinant IL-3; α -IgE, anti-human IgE antibody; γ T, γ -tocopherol.

triplicate. Degranulation was assessed by β -hexosaminidase release (7); total cellular β -hexosaminidase content was quantified by lysing the cells with 0.1% Triton X-100. Supernatants were assayed in duplicate for IL-4, IL-13, and CysLTs using the Luminex 100IS Multiplex flow cytometric assay. Student's t-tests were used to compare γ T-treated cells to the vehicle control.

Pretreatment with 20 μ M γ T modestly but significantly inhibited α -IgE-stimulated β -hexosaminidase release (Fig. 1A, $P = 0.03$). Although there was a significant reduction in IL-4 and IL-13 production ($P < 0.01$ for both) (Fig. 1B–C) compared with the vehicle control, there was a nonsignificant reduction in CysLT production (Fig. 1D, $P = 0.06$). γ T treatment did not induce cellular cytotoxicity compared with the culture media or to the vehicle control (data not shown), assessed by a LDH cytotoxicity assay (Cayman Chemicals, Ann Arbor, MI, USA).

The *ex vivo* study was reviewed and approved by the Committee for the Protection of the Rights of Human subjects of the University of North Carolina and registered on ClinicalTrials.gov (NCT00836368). Twenty *Dermatophagoides farinae*-allergic volunteers (ages 18–50) completed the study. The

primary endpoint was %CD63^{high} basophils as a marker of basophil activation (9) after *ex vivo* challenge with *Der f 1*, using paired testing to compare the postsupplementation values to baseline values.

Each subject took 2 capsules of a γ T-enriched supplement on a daily basis for 7 days provided by YASOO Health Inc. (Johnson City, TN, USA). Each γ T-enriched capsule contained 50 mg d- α -tocopherol, 240 mg d- β tocopherol plus d- δ -tocopherol, and 540 mg d- γ tocopherol (10). Blood was drawn immediately before the first supplementation dose, and 6 h after the day 7 dose. Whole blood was primed with 2 ng/ml of human rIL-3 for 10 min. Samples were then incubated with PBS, 10 μ g/ml α -IgE or *Der f 1* (Greer Laboratories, Lenoir, NC, USA) (0.1, 1, and 10 μ g/ml) for 30 min at 37°C and then placed on ice.

The percentages of CD63^{high} and CD203c^{high} cells served as indices of basophil activation (9). Expression of CD63/LAMP-3 and CD203c/E-NNP3 on circulating basophils was measured by flow cytometry (BD-LSR-II flow cytometer and BD FACSDiva 6.1 software, San Jose, CA, USA). Basophils

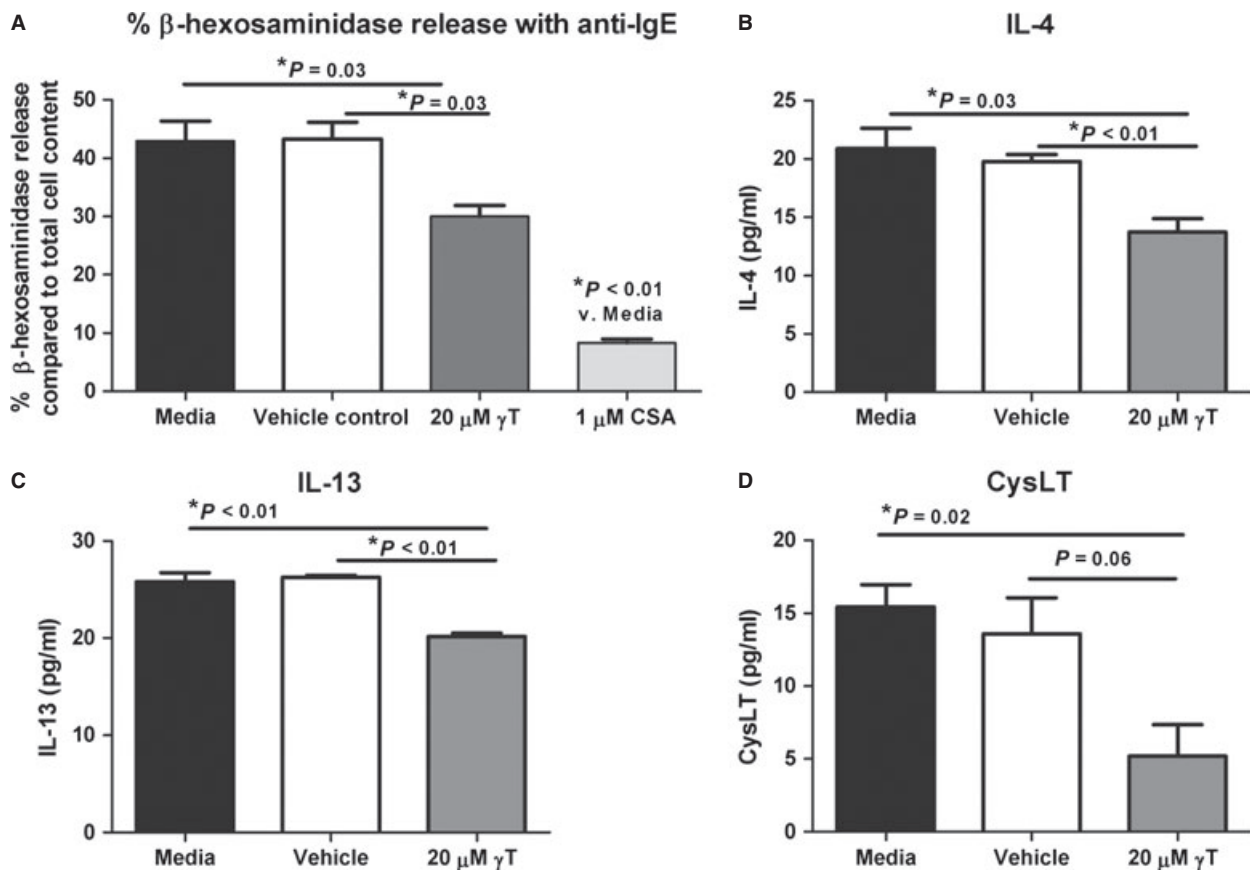


Figure 1 Effect of γ T on degranulation, de novo TH2 cytokines, and cysteinyl leukotrienes in a basophil cell line. (A) % basophil degranulation (compared with total cellular content of β -hexosaminidase) with culture media and with pretreatment with vehicle con-

trol, 20 μ M γ T, or 1 μ M cyclosporine (CSA) prior to stimulation with human α -IgE. De novo production of IL-4 (B), IL-13 (C), and CysLTs (D) after stimulation of RBL SX-38 cells with human α -IgE. Mean and SEM are shown.

were identified in blood as CD203c(+) cells with low side scatter within the CD45(+) leukocyte population. Using histogram analysis, ≈ 98 –99% (minimum 95%) of the basophil population of the un-stimulated controls was gated to establish the baseline expression (mean fluorescent intensity) of each of the surface proteins. Basophils which shifted to the right out of the baseline gate (CD63^{high} and CD203c^{high}) following stimulation with α -IgE or *Der f 1* were considered activated. Flow cytometric values were corrected for mechanical manipulation of cells by subtracting the PBS control values from the α -IgE and *Der f 1* values. Basophil activation was compared before and after 7 days of supplementation using Wilcoxon signed rank tests.

α -IgE and *Der f 1* (0.1, 1, and 10 μ g/ml) all significantly upregulated CD63 and CD203c on basophils compared with the PBS control ($P < 0.001$ for all comparisons). After 1 week of γ T-enriched supplementation, %CD203c^{high} basophils were significantly decreased with α -IgE and all doses of *Der f 1* ($P < 0.01$, Fig. 2A); %CD63^{high} basophils were significantly decreased with α -IgE and the 0.1 μ g/ml *Der f 1* dose (Fig. 2B).

We conducted these studies to determine whether γ T may be a useful adjunct therapy against IgE-mediated processes.

Results of *in vitro* studies suggested IgE-mediated basophil activation, and *de novo* mediator production is modestly reduced with a γ T concentration similar to that observed in the serum of healthy and atopic volunteers after oral supplementation with the identical γ T dosing regimen (10,11). Although supplementation with α -tocopherol was previously noted to increase bleeding risk (12), this side-effect has not been noted with γ T supplementation. We demonstrated that oral consumption of the γ T-enriched supplement by dust mite-allergic volunteers reduced *ex vivo* IgE-mediated upregulation of CD63 and CD203c, suggestive of reduced basophil activation (9). This open-label proof-of-concept study provides rationale for further mechanistic work examining the inhibitory effect of γ T on IgE-mediated activation, as well as for placebo-controlled phase II studies to assess the safety and clinical impact of γ T supplementation as a therapeutic intervention for allergic asthma and rhinitis.

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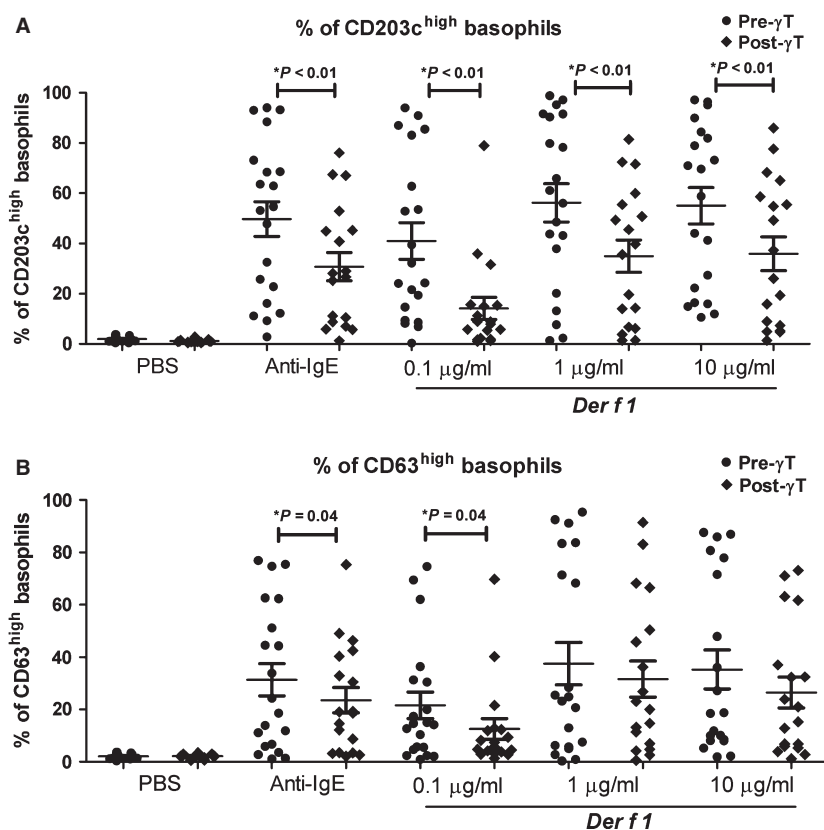


Figure 2 Effect of oral γ T supplementation on basophil activation with dust mite allergen in 20 *Der f 1*-allergic volunteers. Volunteers had positive skin prick testing to *Dermatophagoides farinae* (defined as a 3-mm wheal greater than the negative saline control),

and mean *Der f 1* IgE of 15.8 (SD $\pm$ 21.5) IU/ml. %CD203c^{high} basophils (A) and %CD63^{high} basophils (B) with α -IgE and graded doses of *Der f 1*. Mean and SEM are shown.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

Author contributions

All authors contributed to the design of the study. Experiments were performed by Katherine Mills, John Lay, Weidong Wu, and Matthew Kesic. Carole Robinette prepared the posting for ClinicalTrials.gov, recruited subjects, performed the skin testing and venipuncture, and administered the supplements. Weidong Wu, Katherine Mills, Matthew Kesic, and Michelle Hernandez analyzed the *in vitro* data; Katherine Mills, John Lay, and Matthew Kesic analyzed the *ex vivo* data. Stephen Dreskin facilitated the procurement of the RBL-SX38 cells and provided the methods for both culture and the degranulation experiments. Katherine Mills, Matthew Kesic, David Peden, and Michelle Hernandez wrote the manuscript.

References

1. Schafer T. Epidemiology of complementary alternative medicine for asthma and allergy in Europe and Germany. *Ann Allergy Asthma Immunol* 2004;**93**(2 Suppl 1):S5–S10.
2. Sidora-Arcoleo K, Yoos HL, McMullen A, Kitzman H. Complementary and alternative medicine use in children with asthma: prevalence and sociodemographic profile of users. *J Asthma* 2007;**44**:169–175.
3. Fogarty A, Lewis S, Weiss S, Britton J. Dietary vitamin E, IgE concentrations, and atopy. *Lancet* 2000;**356**:1573–1574.
4. Litonjua AA, Rifas-Shiman SL, Ly NP, Tantisira KG, Rich-Edwards JW, Camargo CA Jr et al. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. *Am J Clin Nutr* 2006;**84**:903–911.
5. Wagner JG, Jiang Q, Harkema JR, Ames BN, Illek B, Roubey RA et al. Gamma-tocopherol prevents airway eosinophilia and mucous cell hyperplasia in experimentally induced allergic rhinitis and asthma. *Clin Exp Allergy* 2008;**38**:501–511.
6. Azzi A, Gysin R, Kempna P, Munteanu A, Negis Y, Villacorta L et al. Vitamin E mediates cell signaling and regulation of gene expression. *Ann N Y Acad Sci* 2004;**1031**:86–95.
7. Palmer GW, Dibbern DA Jr, Burks AW, Bannon GA, Bock SA, Porterfield HS et al. Comparative potency of Ara h 1 and Ara h 2 in immunochemical and functional assays of allergenicity. *Clin Immunol* 2005;**115**:302–312.
8. Blanc F, Adel-Patient K, Drumare MF, Paty E, Wal JM, Bernard H. Capacity of purified peanut allergens to induce degranulation in a functional in vitro assay: Ara h 2 and Ara h 6 are the most efficient elicitors. *Clin Exp Allergy* 2009;**39**:1277–1285.
9. MacGlashan D Jr. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. *Clin Exp Allergy* 2010;**40**:1365–1377.
10. Hernandez ML, Wagner JG, Aline Kala R, Mills K, Wells HB, Alexis NE et al. Vitamin E Gamma-tocopherol reduces airway neutrophil recruitment after inhaled endotoxin challenge in rats and in healthy volunteers. *Free Radic Biol Med* 2013;**60**:56–62.
11. Wiser J, Alexis NE, Jiang Q, Wu W, Robinette C, Roubey R et al. In vivo gamma-tocopherol supplementation decreases systemic oxidative stress and cytokine responses of human monocytes in normal and asthmatic subjects. *Free Radic Biol Med* 2008;**45**:40–49.
12. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 2008;**300**:2123–2133.