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ORIGINAL ARTICLE

Effects of supplementation with tocotrienol-rich fraction on immune response to tetanus toxoid immunization in normal healthy volunteers

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Background/Objectives: Vitamin E is an essential fat-soluble vitamin that has been shown to induce favorable effects on animal and human immune systems. The objective of this study was to assess the effects of tocotrienol-rich fraction (TRF) supplementation on immune response following tetanus toxoid (TT) vaccine challenge in healthy female volunteers.
Subjects/Methods: In this double-blinded, placebo-controlled clinical trial, participants were randomly assigned to receive either placebo (control group) or 400 mg of TRF (study group) supplementation daily. Over the 2-month period of the study, volunteers were asked to attend three clinical sessions (that is, on days 0, 28 and 56) and blood samples were obtained from the volunteers during the follow-up. On day 28, all volunteers were also vaccinated with the TT vaccine (20 Lf) intramuscularly.
Results: The results from the clinical trial showed that TRF supplementation significantly increased the total vitamin E level in the plasma of the TRF-supplemented volunteers compared with the placebo group, indicating overall compliance. Volunteers supplemented with TRF showed a significantly ($P<0.05$) enhanced production of interferon-γ and interleukin (IL)-4 by the mitogen or TT-stimulated leukocytes compared with the control group. Volunteers from the TRF group produced significantly ($P<0.05$) lower amounts of IL-6 compared with the placebo group. Anti-TT IgG production was also significantly ($P<0.05$) augmented in the TRF-supplemented group compared with the placebo group.
Conclusions: We conclude that TRF has immunostimulatory effects and potential clinical benefits to enhance immune response to vaccines.
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Keywords: tocotrienol-rich fraction; tetanus toxoid; immunostimulatory

Introduction

Tocopherols and tocotrienols are bioactive plant derivatives that belong to the vitamin E family. The latter are found abundantly in palm oil, consisting mainly of α-, β-, γ- and δ-tocotrienol (Sundram and Gapor, 1992). It has been reported that tocotrienols have many beneficial health effects that are often not exhibited by tocopherols, such as anti-cancer (Nesaretnam *et al*., 2000, 2004) and neuroprotective (Sen *et al*., 2000) properties. To date, there have been several studies highlighting the use of tocopherols to enhance the immune system in elderly human subjects (Meydani *et al*., 1997a, b), aged mice (Meydani *et al*., 1997a, b) and Brown Norway rats (Gu *et al*., 1999). However, the effects of tocotrienol on the human immune system following immunization have not been investigated.

Activated T cells can differentiate into effector T cells showing distinct patterns of cytokine production. The T-helper-1 (T_H1) cells, which regulate cell-mediated immune response, produce interferon (IFN)-γ, while the T_H2 cells, which mediate humoral immune responses, differentiate by their production of interleukin (IL)-4 and IL-5 (Mosmann and Coffman, 1989). Immunization with a potent immunogen such as the tetanus toxoid (TT) antigen can induce long-lasting immunity in humans (Kilian and Nielsen, 1980). Immune response against tetanus is generally associated with the production of neutralizing IgG antibodies to TT (Kilian and Nielsen, 1980).

In this study, we evaluated the effects of 400 mg of tocotrienol-rich fraction (TRF) supplementations on

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immune response and modulation following a booster TT vaccination in healthy female volunteers.

Materials and methods

Study design
This study, a randomized, double-blinded, placebo-controlled trial, was approved by the Research and Ethics Committees of the International Medical University and followed the Malaysian guidelines for Good Clinical Practice. The 108 healthy women (18–25 years) recruited were randomly assigned into control (placebo) or experimental (400 mg of TRF daily) groups. Written informed consent was obtained from all the volunteers. Before recruitment, the volunteers were screened to ensure that they met the study's inclusion (non-smoker, healthy, no major organ failures, not on any treatment, not taken vitamin E supplements in the last 3 months, not pregnant, had no TT vaccine in the last 5 years) and exclusion criteria. As the study required non-smokers, only female volunteers were recruited. It should be noted that all volunteers had received TT vaccination as part of their post-natal vaccination regime. The volunteers were asked to attend three clinic sessions on days 0, 28 and 56. During these sessions, their blood was taken for various tests related to the study. During the period of the study, volunteers were given three meals a day with a standard recommended diet schedule in order to keep the calorie intake constant and to minimize the effects of diet on the overall absorption of vitamin E. Compliance was checked by pill counts at each visit.

Tocotrienol-rich fraction
The TRF supplements used in this study were Tocovid SupraBio, manufactured by Hovid Sdn Bhd (Ipoh, Malaysia). The composition of Tocovid soft gel capsules includes a mixture of tocotrienols and α-tocopherols (α-T's) (Table 1). Each TRF capsule contained 200 mg of the supplement, while the placebos were made of soy oil. Both the supplements were identical in weight and appearance. Volunteers were asked to take two tablets per day of their supplements (TRF or placebo) for 56 days, preferably with lunch or dinner. This dose (400 mg per day) was chosen based on the other studies that used vitamin E supplementation (Kappus and Diplock, 1992; Meydani *et al*., 1997a, b).

Table 1 Tocotrienol and tocopherol composition of TRF supplement

Vitamin E	*Composition (per tablet)*
D-α-tocotrienol	61.52 mg
D-γ-tocotrienol	112.80 mg
D-δ-tocotrienol	25.68 mg
D-α-tocopherol	91.60 IU

Abbreviation: TRF, tocotrienol-rich fraction.

TT vaccination
On day 28 of the study, all volunteers received a single dose of TT vaccine. The TT vaccine (Biofarma, Bandung, Indonesia) administered through intramuscular injection of TT vaccine (20 flocculation units) into the deltoid muscle of the patient's non-dominant arm by a registered nurse. There were no reports of any vaccine-related serious adverse events.

Isolation of human blood leukocytes
Human leukocytes were isolated from whole blood using a red blood cell lysis buffer (eBioscience, San Diego, CA, USA) following the manufacturer's protocol. The recovered peripheral blood leukocytes (PBLs) were resuspended in 5 ml of RPMI-1640 containing 5% (v/v) of fetal bovine serum, 1% of penicillin, streptomycin and L-glutamine. The tubes were kept on ice and cell count was performed using a hemocytometer.

Proliferation of blood leukocytes
Leukocytes were seeded in a 96-well plate at 1×10^6 cells/well and the cells were stimulated individually with either 1 μg/ml of Concanavalin A (Con A) (Sigma-Aldrich, St Louis, MO, USA) or lipopolysaccharide (Sigma-Aldrich) or 10 μg/ml of TT (Calbiochem, San Diego, CA, USA). The cells were cultured for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The culture supernatant was harvested after 3 days and stored at −80 °C.

Enzyme-linked immunosorbent assay
The amounts of IFN-γ, IL-4, IL-6 and IL-10 in the culture supernatants of human leukocytes were estimated using commercial ELISA kits (eBioscience) according to the manufacturer's instructions. The limit of sensitivity for detection of IFN-γ and IL-6 was 8 and 1 pg/ml, respectively. The detection limit for both IL-4 and IL-10 was 4 pg/ml. The anti-TT IgG (IU/ml) in the plasma samples was quantified using the Anti-Tetanus Human IgG ELISA kit according to the manufacturer's recommended protocol (IBL, Hamburg, Germany).

Vitamin E extraction from blood plasma and HPLC analysis
Plasma was isolated from whole blood. About 500 μl of plasma was added to a tube containing 0.5 ml of 0.5% NaCl, ethanol and 400 μl of hexane. The mixture was shaken vigorously for an hour using a minishaker. The tubes were then spun at 3000 r.p.m. for 10 min at room temperature. After centrifugation, the clear hexane phase was transferred carefully into a clean vial and blow-dried under nitrogen gas. An aliquot of the lipid sample was reconstituted in 500 μl hexane. Approximately 10 μl of the sample and a standard solution mixture containing α-T's, α-, γ- and δ-tocotrienols was also injected accordingly into the HPLC system.

The excitation wavelength and emission wavelength of the fluorescence detector were set at 295 and 325 nm, respectively. The mobile phase was hexane-isopropyl alcohol (99.5/0.5, v/v) with a flow rate of 2 ml/min. The peak areas of the components in the sample were compared with those of the standards and used for the quantitative calculation.

Statistical analysis

Data obtained during the study were processed using SPSS for Windows (Version 18.0; SPSS Inc., Chicago, IL, USA). The repeated measures test, which is a test that is available under the general linear model analysis in the SPSS software version 18, was used to determine the significance between the control (placebo) and experimental (TRF) groups at three different time points (that is, on days 0, 28 and 56). Data are presented as mean ± s.d.

Results

IgG levels in plasma following TRF supplementation and TT vaccination

An enhanced anti-TT IgG production was also observed in the TRF-supplemented group after TT –vaccination (that is, on day 56; Figure 1). The mean level of anti-TT IgG in the study population before the TT vaccination was 0.79 IU/ml and the levels increased significantly ($P<0.05$) 1 month after the TT vaccination in both TRF- and placebo-supplemented volunteers. On day 56, the mean anti-TT IgG levels in the placebo- and TRF-supplemented groups were 1.03 and 1.93 IU/ml, respectively, and the levels were significant ($P<0.05$) in the TRF group as compared with the placebo group (Figure 1). Volunteers in both groups had a protective anti-TT response after vaccination, which is defined as anti-tetanus antibody level of $\geqslant 0.85$ IU/ml (Kilian and Nielsen, 1980).

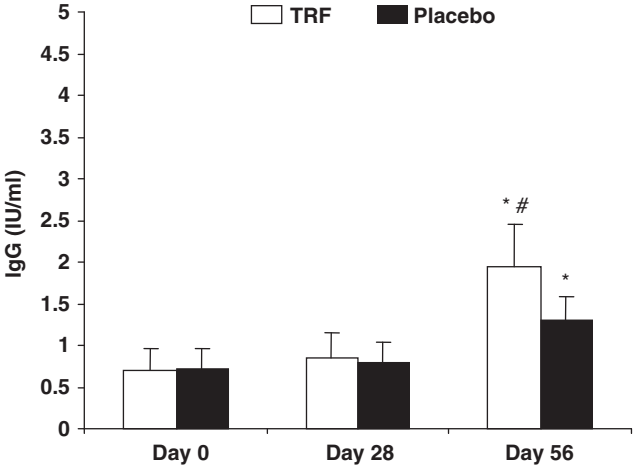

Figure 1 Anti-TT IgG concentrations in the plasma of TRF- and placebo-supplemented volunteers. The Greenhouse–Geisser method was used to test for Day and Day × Group interaction effect. The results showed that there was significant difference in the production of IFN-γ between day 0 and day 56 *($F=120.338$, d.f. $=1.35$, $P<0.001$, partial $\eta^2=0.572$, power $=100\%$). There was also a sizeable Day × Group interaction effect #($F=13.611$, d.f. $=1.35$, $P<0.001$, partial $\eta^2=0.131$, power $=98.4\%$).

Effect of TRF supplementation on the production of IFN-γ by Con A- or TT-stimulated blood leukocytes

The production of IFN-γ by PBLs following Con A stimulation was significantly ($P<0.05$) enhanced on day 56, that is, after TT vaccination, when compared with that produced on day 0 or 28, and the cytokine level was significantly augmented ($P<0.05$) in the TRF-supplemented group as compared with the placebo group (Figure 2a). On day 28, before the TT immunization, TT-specific IFN-γ production was significantly ($P<0.05$) higher in the TRF-supplemented volunteers compared with the placebo group (Figure 2b).

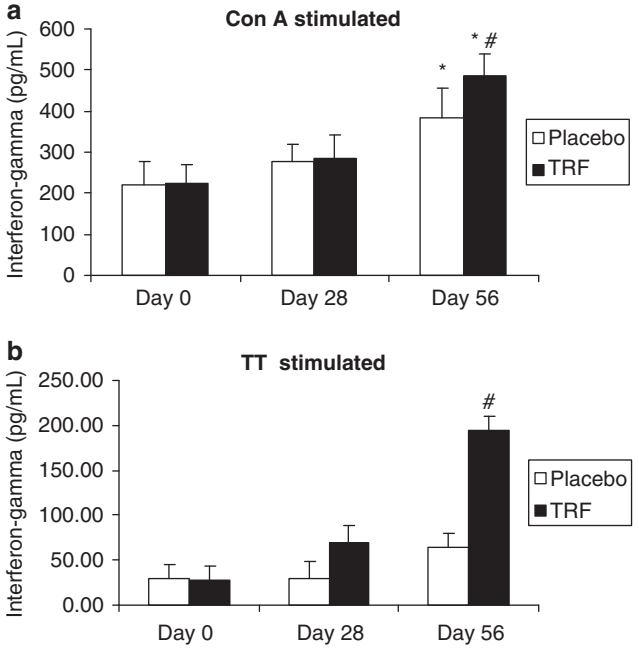

Figure 2 Production of IFN-γ by Con A (**a**) and TT (**b**) stimulated PBLs from TRF- and placebo-supplemented volunteers. (**a**) Con A: The Greenhouse–Geisser method was used to test for Day and Day × Group interaction effect. The results showed that there was significant difference in the production of IFN-γ between day 0 and day 56 *($F=358.932$, d.f. $=1.6$, $P<0.001$, partial $\eta^2=0.79$, power $=100\%$). There was also a sizeable Day × Group interaction effect #($F=21.914$, d.f. $=1.6$, $P<0.001$, partial $\eta^2=0.189$, power $=100\%$). (**b**) TT: The Greenhouse–Geisser method was used to test for Day and Day × Group interaction effect. The results showed that there was significant difference between the production of IFN-γ between day 0 and day 56 *($F=26.935$, d.f. $=1$, $P<0.001$, $\eta^2=0.627$, power $=99.9\%$). There was also a sizeable Day × Group interaction effect #($F=15.129$, d.f. $=1$, $P<0.001$, $\eta^2=0.486$, power $=95.4$ in panel b of figure 2.%).

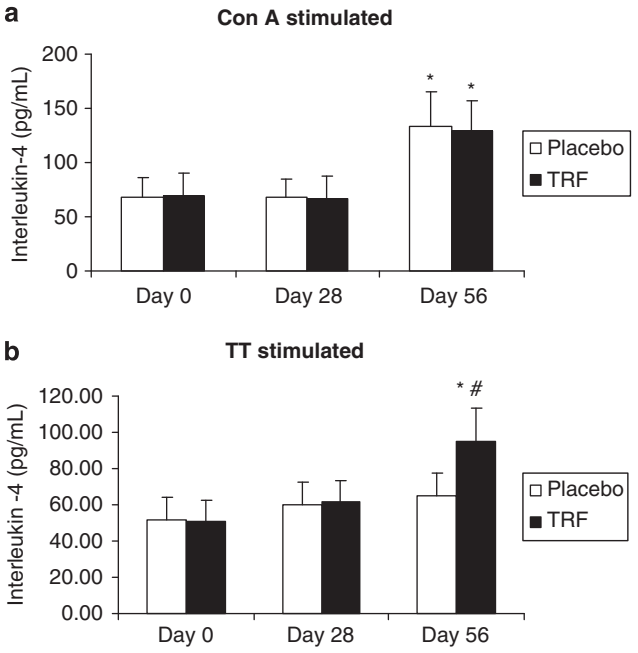

Figure 3 Production of IL-4 by Con A (**a**) and TT (**b**) stimulated PBLs in volunteers supplemented with TRF and placebo. (**a**) Con A: The Greenhouse–Geisser method was used to test for Day and Day × Group interaction effect. The results showed that there was significant difference in the production of IL-4 between day 0 and day 56 *($F = 358.932$, d.f. = 1.6, $P < 0.001$, partial $\eta^2 = 0.79$, power = 100%). (**b**) TT: The Greenhouse–Geisser method was used to test for Day and Day × Group interaction effect. The results showed that there was significant difference in the production of IL-4 between day 0 and day 56 *($F = 120.338$, d.f. = 1.35, $P < 0.001$, partial $\eta^2 = 0.572$, power = 100%). There was also a sizeable Day × Group interaction effect #($F = 13.611$, d.f. = 1.35, $P < 0.001$, partial $\eta^2 = 0.131$, power = 98.4%).

This could be due to the previous exposure to this antigen during childhood immunization programs. The production of IFN-γ on day 56 due to TT stimulation was also significantly higher ($P < 0.05$) in the TRF-supplemented volunteers (Figure 2b).

Effect of TRF supplementation on the production of IL-4 by Con A- or TT-stimulated PBLs

Before the TT vaccination, PBLs of the volunteers produced very low levels of IL-4 in Con A-stimulated cultures (Figure 3a). At 1 month after the TT vaccination, the levels of IL-4 were significantly ($P < 0.05$) elevated in both TRF- and placebo-supplemented groups as compared with day 28. However, the concentration of IL-4 on day 56 did not differ significantly ($P > 0.05$) between the control and experimental groups. The IL-4 levels in TT-stimulated blood leukocyte cultures were assayed at two time points, that is, at 4 weeks pre- (day 28) and post-TT vaccination (day 56). Blood leukocytes stimulated by TT produced lower amounts of

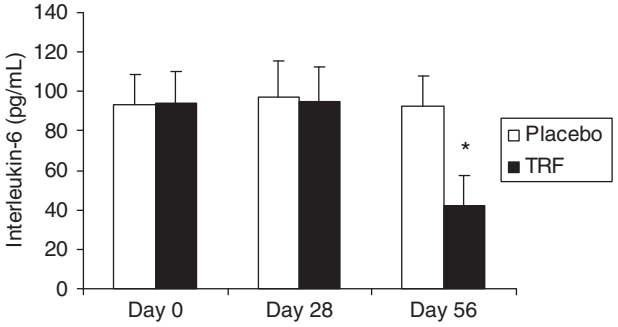

Figure 4 Concentration of IL-6 following lipopolysaccharide stimulation in the PBLs of volunteers supplemented with TRF and placebo (significant difference: *$P < 0.05$ between TRF and placebo groups on day 56).

IL-4 as compared with Con A-stimulated blood leukocytes (Figures 3a and b). Before the TT vaccination (that is, on day 28), there was no statistically significant ($P > 0.05$) difference in the amount of IL-4 produced by TRF- or placebo-supplemented volunteers (Figure 3b) following TT stimulation. However, on day 56, the IL-4 level was significantly ($P < 0.05$) augmented following TT vaccination in the volunteers who received TRF as compared with placebo (Figure 3b).

Effect of TRF supplementation on the production of IL-6 by lipopolysaccharide-stimulated blood leukocytes

Pro-inflammatory cytokines such as IL-6 and TNF-α are produced by macrophages to induce acute-phase proteins following pathogenic challenge (Alcami and Koszinowski, 2000). The lipopolysaccharide-stimulated blood leukocytes from the volunteers produced high levels of IL-6 on days 0 and 28 of this study (Figure 4) in both the control and experimental groups. However, the volunteers supplemented with TRF had significantly ($P < 0.05$) lower production of IL-6 on day 56, that is, 1 month after the TT immunization in comparison with the volunteers who received placebo.

High levels of Vitamin E in the plasma of volunteers supplemented with TRF

It has been reported that plasma α-T levels are 10-fold higher than the levels of other tocopherol or tocotrienol isomers (Jiang *et al*., 2001). The amount of endogenous α-T in the blood of healthy volunteers increased significantly ($P < 0.05$) on days 28 and 56 as compared with day 0 (Figure 5a). The α-T concentration was significantly ($P < 0.05$) augmented in the TRF-supplemented group compared with the placebo group on days 28 and 56. There were no significant differences ($P > 0.05$) in the γ- and δ-tocopherol concentrations between control and placebo (Figures 5b and c). Mean plasma α-, γ- and δ-tocotrienol concentrations also increased significantly ($P < 0.05$) in the volunteers who received TRF

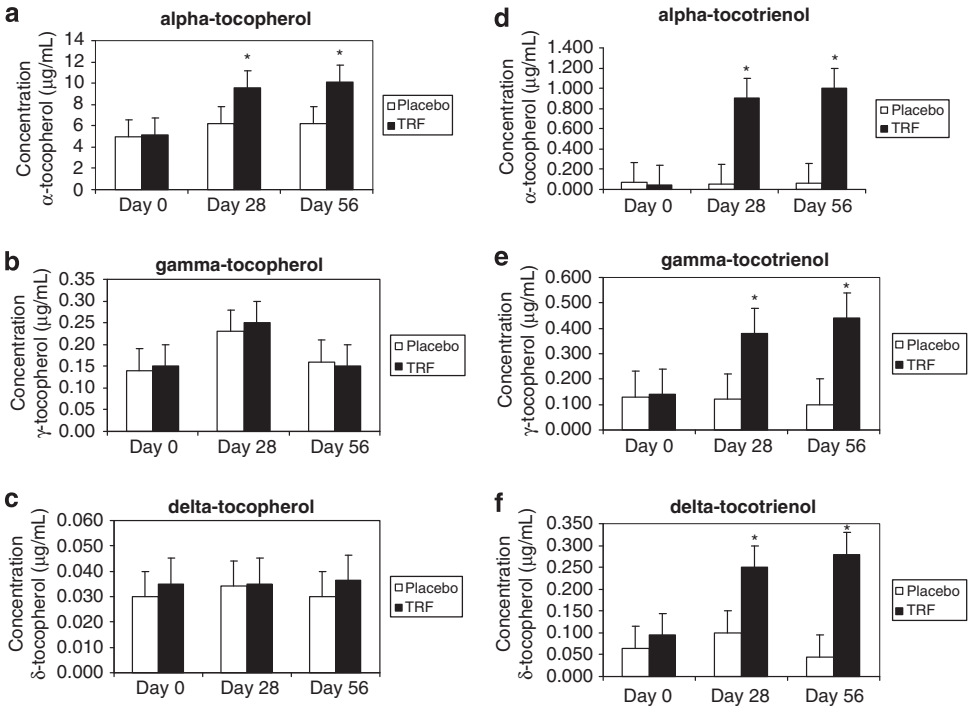

Figure 5 Concentrations of vitamin E in the plasma of control (placebo) and TRF-supplemented volunteers. Plasma levels of α-T (**a**), γ-tocopherol (**b**), δ-tocopherol (**c**), α-tocotrienol (**d**), γ-tocotrienol (**e**) and δ-tocotrienol (**f**) are shown at days 0, 28 and 56 of the study. Statistical analysis showed that there was significant difference in plasma levels of α-T ($F = 27.974$, d.f. = 1, $P < 0.01$, partial $\eta^2 = 0.222$, power = 99.9%); α-tocotrienol *($F = 15.861$, d.f. = 1.625, $P < 0.01$, partial $\eta^2 = 0.139$, power = 99.8%); γ-tocotrienol ($F = 8.375$, d.f. = 1.603, $P < 0.01$, partial $\eta^2 = 0.079$, power = 92.8%); δ-tocotrienol, ($F = 26.365$, d.f. = 1, $P < 0.01$, partial $\eta^2 = 0.919$, power = 100%) between days 0 and 28 and between days 0 and 56 in the control and TRF-supplemented groups.

compared with placebo on days 28 and 56 (Figures 5d–f). Among the tocotrienol isomers in the TRF-supplemented group on days 28 and 56, the concentration of α-tocotrienol was the highest and this was followed by γ- and δ-tocotrienol (Figures 5d–f). However, the concentrations of tocotrienols in the placebo group on days 28 and 56 remained the same and the amounts did not differ significantly ($P > 0.05$) compared with day 0. As for β-tocopherols and tocotrienols, the concentrations were low and almost undetectable in plasma.

Discussion

Improvement of cell-mediated immunity by vitamin E has been extensively documented in animal studies (Bendich *et al*., 1986; Moriguchi *et al*., 1990; Meydani *et al*., 1997a, b; Vatassery *et al*., 1999). Dietary vitamin E supplementation has been shown to increase IL-2 production as well as lymphocyte proliferation in experimental animals (Bendich *et al*., 1986; Moriguchi *et al*., 1990). The existing literature is less clear on the suggested benefits of dietary supplementation of vitamin E for immune functions in healthy well-nourished subjects, with no overt signs of a compromised immune system. Vitamins with antioxidant activities are known to enhance immune response (Park *et al*., 2003). Vitamin E is a strong antioxidant that can support monocyte/macrophage-mediated responses (Tits van *et al*., 2000). Vitamin has also been reported to increase delayed type hypersensitivity and T-cell responses in healthy elderly people (Meydani *et al*., 1997a, b; Pallast *et al*., 1999).

There is less information known on how vitamin E, particularly tocotrienols, affects the functional activities of human immune cells following a vaccine challenge. In this study we looked to see whether daily supplementation of 400 mg of TRF for 2 months could enhance the immune response in human volunteers challenged with TT vaccine. The dose of TRF (400 mg) chosen for this study is in line with the other vitamin E studies (Meydani *et al*., 1997a, b; Yusuf *et al*., 2000; Yu *et al*., 2006). Recently, we reported that supplementation of α-T or TRF for 2 months did not produce any significant changes on immune parameters in healthy human subjects in the absence of immunogenic challenge (Radhakrishnan *et al*., 2008). Hence, in this study, in addition to the TRF supplementation, we have also chosen to vaccinate the volunteers with a TT vaccine. This vaccine was chosen as it is a well-characterized and potent immunogen (Sneath *et al*., 1987) and the ability to mount recall immune responses to TT is considered to be indicative of a healthy and immunocompetent immune system (Vatassery *et al*., 1999).

In this study, before the booster TT vaccination, nearly all participants were well protected against this antigen (that is, anti-TT titers were $\geqslant$0.15 IU/ml). At 1 month after the TT vaccination (day 56), the anti-TT IgG levels increased and the maximum was reached in the TRF-supplemented group (1.93 IU/ml) compared with the participants who received placebo (1.03 IU/ml). Our results are also in agreement with the findings of Meydani *et al*. (1997a, b), who demonstrated that vitamin E enhances antibody response to tetanus and hepatitis-B following the vaccinations in healthy elderly subjects.

The level of antigen-specific IFN-γ production by PBL has been used by previous studies as an indicator of cellular immunity (Bendich *et al*., 1986; Alcami and Koszinowski, 2000). In this paper, we show that Con A and TT stimulation of the PBL from the TRF-supplemented group produced significantly higher amounts of IFN-γ on day 56 compared with the placebo group. In addition, the level was higher than that produced on days 0 and 28 of this study. In addition, the PBL from the TRF-supplemented volunteers also increased IFN-γ production on day 28, that is, before the TT vaccination. As all volunteers recruited in this study have been vaccinated with TT as part of their routine vaccination after birth, this observation was not a surprise. There was only a weak production of IL-4, a T_H2-cytokine, by the PBL from the TRF-supplemented volunteers. This observation is consistent with previous report that vitamin E supplementation augmented production of T_H1 cytokines but not T_H2 cytokines in healthy elderly subjects ((Meydani *et al*., 1997a, b).

Immunizations are known to induce acute-phase response provoked by the mild inflammatory challenge of vaccination (De Waart *et al*., 1997). In this study, IL-6, an inflammatory cytokine, although in the normal range (Bruunsgaard *et al*., 1999), was elevated before vaccination in both TRF and placebo groups. However, the cytokine secretion was significantly reduced 1 month after the TT vaccination (day 56) in the TRF-supplemented group as compared with placebo.

Analysis of plasma vitamin E levels of the volunteers showed that the TRF-supplemented volunteers had significantly higher concentrations of α-T, α-T_3, γ-T_3 and δ-T_3 in their plasma on days 28 and 56 as compared with day 0 of the study or the volunteers who received placebo capsules. Tocotrienols constitute up to 70% of the TRF (Sundram and Gapor, 1992). α-T made up the remaining 30% of the TRF supplement. Plasma α-T levels were 9–10-fold higher than the tocotrienol isomers. This could be because of the presence of α-T transfer protein together with tocopherol-associated proteins in the plasma that have been reported to be responsible for the endogenous accumulation of natural α-T (Jiang *et al*., 2001). Among the T_3 tocotrienols, α-T_3 had the highest absorption, followed by γ-T_3 and δ-T_3 in all the individuals supplemented with TRF on days 28 and 56 as compared with the placebo group and basal (on day 0). However, levels of tocotrienols appeared to be significantly lower when compared with the concentration of α-T in the blood plasma after 6 h of ingestion of the TRF capsule. The bioavailability of the orally taken tocotrienols is relatively less compared with that of α-T. However, although the affinity to transport tocotrienols is much lower, it has been shown that α-tocotrienol at nanomolar concentrations can protect neurons against a range of neurotoxic insults (Sen *et al*., 2000). This suggests that low levels of the circulating tocotrienol may be able to regulate immune function favorably.

In conclusion, this study provides evidence that daily supplementation of 400 mg TRF can have a beneficial role in enhancing the immune response of healthy subjects following an immunogenic challenge such as vaccination.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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References

Alcami A, Koszinowski UH (2000). Viral mechanisms of immune evasion. *Immunol Today* **21**, 447–455.

Bendich A, Gabriel E, Machlin LJ (1986). Dietary vitamin E requirement for optimum immune response in the rat. *J Nutr* **116**, 675–681.

Bruunsgaard H, Pedersen AN, Schroll M, Skinhoj P, Pedersen BK (1999). Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. *Clin Exp Immunol* **118**, 235–241.

De Waart FG, Portengen L, Doekes G, Verwaal CG, Kok FJ (1997). Effect of 3 months vitamin E supplementation on indices of the cellular and humoral immune response in elderly subjects. *Br J Nutr* **8**, 761–774.

Gu JY, Wakizono Y, Sunada Y, Hung P, Nonaka M, Sugano M *et al*. (1999). Dietary effect of tocopherols and tocotrienols on the immune function of spleen and mesenteric lymph node lymphocytes in Brown Norway rats. *Biosci Biotechnol Biochem* **63**, 1697–1702.

Jiang Q, Christen S, Shigenaga MK, Ames BN (2001). γ-tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am J Clini Nutr* **74**, 714–722.

Kappus H, Diplock AT (1992). Tolerance and safety of vitamin E: a toxicological position report. *Free Radic Biol Med* **13**, 55–74.

Kilian HD, Nielsen G (1980). Cell-mediated and humoral immune response to tetanus vaccinations in onchocerciasis patients. *Ann Trop Med Parasitol* **40**, 285–291.

Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R *et al.* (1997a). Vitamin E supplementation and *in vivo* immune

response in healthy elderly subjects. A randomized controlled trial. *J Am Med Assoc* **277**, 1380–1386.
Meydani SN, Meydani M, Verdon CP, Shapiro AC, Blumberg JB, Hayes KC (1997b). Vitamin E supplementation suppresses prostaglandin E2 synthesis and enhances the immune response of aged mice. *Mech Ageing Dev* **34**, 191–201.
Moriguchi S, Kobayashi N, Kishino Y (1990). High dietary intakes of vitamin E and cellular immune functions in rats. *JNutr* **120**, 1096–1102.
Mosmann TR, Coffman RL (1989). Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* **7**, 145–173.
Nesaretnam K, Ambra R, Selvaduray KR, Radhakrishnan A, Canali R, Virgili F (2004). Tocotrienol-rich fraction from palm oil and gene expression in human breast cancer cells. *AmnN Y Acad Sci* **1031**, 143–157.
Nesaretnam K, Dorasamy S, Darbre PD (2000). Tocotrienols inhibit growth of ZR-75-1 breast cancer cells. *Int J Food Sci Nutr* **51**, S97–103.
Pallast EG, Schouten EG, De Waart FG, Fonk HC, Doekes G, von Blomberg BM *et al*. (1999). Effect of 50- and 100-mg vitamin E supplements on cellular immune function in noninstitutionalized elderly persons. *Am J Clin Nutr* **69**, 1273–1281.
Park OJ, Kim HY, Kim WK, Kim YJ, Kim SH (2003). Effect of vitamin E supplementation on antioxidant defense systems and humoral immune responses in young, middle-aged and elderly Korean women. *J Nutr Sci Vitaminol* **49**, 94–99.
Radhakrishnan AK, Lee AL, Wong PK, Kaur J, Aung H, Nesaretnam K (2008). Comparable effects on immune modulation following daily supplementation with tocotrienol- rich fraction (TRF) or alpha-tocopherol in normal human volunteers. *Br JNutr* **101**, 810–815.
Sen CK, Kanna S, Roy S, Packer L (2000). Molecular basis of Vitamin E action. *J Biol Chem* **275**, 13049–13055.
Sneath PAT, Kerslake EG, Scruby F (1987). Tetanus immunity: the resistance of guinea pigs to lethal spore doses induced by active and passive immunization. *Am J Trop Med Hyg* **25**, 464–476.
Sundram K, Gapor A (1992). Vitamin E from Palm Oil; Its Extraction and Nutritional Properties. *Lipid Technol* **7**, 137–141.
Tits van LJ, Demacker PN, Graaf JD, Hak-Lemmers HK, Stalenhoef AF (2000). Alpha-tocopherol supplementation decreases production of superoxide and cytokines by leukocytes *ex vivo* in both normolipidemic and hypertriglyceridemic individuals. *Am J Clin Nutr* **77**, 458–464.
Vatassery GT, Bauer T, Dysken M (1999). High doses of vitamin E in the treatment of disorders of the central nervous system in the aged. *Am J Clin Nutr* **70**, 793–801.
Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P (2000). Vitamin E supplementation and cardiovascular events in high risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* **342**, 154–160.
Yu SG, Thomas AM, Gapor A, Tan B, Qureshi N, Qureshi AA (2006). Dose-response impact of various tocotrienols on serum lipid parameters in 5-week-old female chickens. *Lipids* **41**, 453–461.