The Modulating Effect of Vitamin E on Active Oxygen Generation by Alveolar Macrophage Challenged with Different Stimuli

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We investigated the effect of vitamin E on active oxygen generation by alveolar macrophages (AMs) challenged with different stimuli to determine the influence of the vitamin. AMs were obtained from rats orally treated with vitamin E (100 mg/kg or 150 mg/kg) for 6 d, and Concanavalin A (Con A) and 12-*o*-tetradecanoylphorbol-13-acetate (TPA) were used as stimuli. AMs from the vitamin E-treated group showed a dose-dependent increase of active oxygen generation when stimulated with Con A, while a dose-dependent decrease of production by AMs challenged with TPA was observed with the vitamin treatment. These results suggest that vitamin E has a modulating effect on active oxygen production by AMs stimulated with stimuli.

Keywords — modulating effect ; vitamin E ; active oxygen ; alveolar macrophage ; stimulus ; Concanavalin A

Introduction

Phagocytes like macrophage generate active oxygen species by activation of NADPH oxidase systems in response to extracellular stimuli.¹⁾ Such active oxygen species then play an important role in reducing incidences of tumors and infection.²⁾ At the beginning of the host defense in lung, alveolar macrophages (AMs) play a central role.³⁾ Moreover, it has been reported that vitamin E has modulating effects on cellular functions in phagocytes,⁴⁾ but the influence of vitamin E on active oxygen production by AMs has not been investigated in detail. We report the study of the effect of this vitamin E on the important active oxygen generation function of AMs challenged with Concanavalin A (Con A) or 12-*o*-tetradecanoylphorbol-13-acetate (TPA).

Con A binds to saccharide moieties on the surface of phagocytes and can trigger active oxygen production by mobilizing of Ca^{2+} from

the extracellular Ca^{2+} pool.^{5a,b)} TPA can directly activate protein kinase C (PKC) and induce active oxygen generation by phagocytes.⁶⁾ In addition, PMA does not elicit any increase in cytosolic calcium;⁷⁾ for this reason we used Con A and TPA as stimuli.

Materials and Methods

Animals and Treatment — Eight week-old, male rats of a specific pathogen-free Sprague-Dawley strain (Japan Clea, Tokyo, Japan) were used. The rats were fed a diet (CE-2, Japan Clea) and sterilized water ad libitum. They were orally treated with DL- α -tocopherol (100 mg/kg or 150 mg/kg, Tokyo Kasei, Tokyo, Japan) in a suspension of soybean oil (Wako Pure Chemicals, Osaka, Japan) for 6 d.

Cell Preparation — All rats were sacrificed under anesthesia with pentobarbital at 24 h after final treatment of vitamin E. AMs were prepared by pulmonary lavage using a previous method,⁸⁾ then suspended in Hank's balanced salt solution (phenol red-free) and stored at 4°C. AMs

were more than 95% pure and 98% of the cells exclude trypan blue.

Assay of Active Oxygen Production — To monitor active oxygen production by AMs, lucigenin (Boehringer, Mannheim, German)-amplified chemiluminescence was measured at 37°C by a luminescence reader (Aloka, Tokyo, Japan).⁹⁾ In this assay, 1×10^6 AMs were used in a final volume of 1 ml in the presence of 250 μ M lucigenin. To induce a respiratory burst of AMs, Con A and TPA as stimuli were used at final concentration of 100 μ g/ml and 10 ng/ml, respectively.

Measurement of α -Tocopherol Level — The level of α -tocopherol in AMs and serum was determined by high performance liquid chromatography.¹⁰⁾

Statistical Analysis — Statistical comparisons between control and vitamin E-treated groups were performed using Student's or Welch's *t*-test after analysis of variance. $p < 0.05$ was used for significant difference.

Results

The effect of vitamin E on total active

oxygen generation by AMs is shown in Table I. Vitamin E dose-dependent increase of active oxygen generation by AMs stimulated with Con A was observed. The values in 100 mg/kg and 150 mg/kg vitamin E-treated groups statistically increased by 50% and 91% in comparison with the value in control. On the other hand, active oxygen formation by AMs stimulated with TPA showed a vitamin E dose-dependent decrease with significant difference. The values in 100 mg/kg and 150 mg/kg vitamin E-treated groups showed 59% and 67% decreases as compared to the value in control, respectively.

α -Tocopherol level in serum indicated a dose-dependent increase following vitamin E treatment for 6 d (Table II): the levels in control, 100 mg/kg and 150 mg/kg vitamin E-treated groups were 5.20 ± 0.46 μ g/ml, 8.92 ± 1.05 μ g/ml and 9.54 ± 1.06 μ g/ml, respectively. Similarly, α -tocopherol content in AMs of rats treated with 100 mg/kg vitamin E statistically increased by 98% as compared to the content in control (Table II).

TABLE I. Effect of Vitamin E Treatment on Lucigenin-dependent Chemiluminescence by Con A or TPA-stimulated AMs in Rats^{a)}

Stimulant	Active oxygen formed (kcount/ 10^6 cells)		
	Control	Vitamin E (100 mg/kg)	Vitamin E (150 mg/kg)
Con A	0.418 ± 0.032	$0.666 \pm 0.037^b)$	$0.791 \pm 0.091^b)$
TPA	0.721 ± 0.121	$0.295 \pm 0.017^b)$	$0.236 \pm 0.012^b)$

a) 1×10^6 AMs were assayed in a total volume of 1 ml with 250 μ M lucigenin, and Con A (100 μ g/ml) or TPA (10 ng/ml) was used as a stimulant. The recording of chemiluminescence by AMs stimulated with Con A or TPA was performed for 30 min after the addition of stimulant. Values are expressed as the mean $\pm$ S.E. from 4 rats. b) significant difference with respect to control.

TABLE II. Content of α -Tocopherol in Serum and AMs from Vitamin E-treated Rats^{a)}

Fraction	α -Tocopherol content ^{b)}		
	Control	Vitamin E (100 mg/kg)	Vitamin E (150 mg/kg)
Serum	5.20 ± 0.46	$8.92 \pm 1.05^c)$	$9.54 \pm 1.06^c)$
AMs	159.1 ± 14.8	$315.7 \pm 45.8^c)$	N.T. ^{d)}

a) Values are expressed as the mean $\pm$ S.E. from 4 rats in serum and 4 samples in AMs. One sample was obtained from 3 rats. b) α -Tocopherol content in serum (μ g/ml); α -tocopherol content in AMs (ng/ 10^6 cells). c) Significant difference with respect to control. d) N.T., not tested.

Discussion

It was indicated in this study that vitamin E reinforced active oxygen production by Con A-stimulated AMs and inversely attenuated this production by TPA-stimulated AMs. These phenomena could presumably be dependent on the increase of α -tocopherol level in AMs due to the vitamin treatment, because α -tocopherol level in AMs and serum showed a similar increase in response to vitamin E, and active oxygen production by Con A-stimulated AMs had a positive correlation with α -tocopherol level in serum ($r=0.762$, data not shown).

TPA is known to activate protein kinase C (PKC) directly and to induce respiratory burst in phagocytes.⁶⁾ On the other hand, it has been reported that PKC activity can be inhibited by vitamin E treatment in some cells.¹¹⁾ Thus, the inhibition of active oxygen production by TPA-stimulated AMs from rats treated with vitamin E in this study presumably depends on the inhibition of PKC activation associated with the increase of α -tocopherol level in AMs.

Con A is believed to bind to saccharide moieties on the cell surface,^{5b)} and the main signal inducing respiratory burst in phagocytes is calcium-dependent pathways.¹²⁾ In our preliminary experiment, the levels of intracellular Ca^{2+} in AMs stimulated with Con A between control and vitamin E-treated groups were similar, suggesting that a

calcium-dependent pathway like the calcium-calmodulin-mediated pathway¹³⁾ could not contribute to the augmentation of active oxygen formation in Con A-stimulated AMs. It is also suggested that calcium-independent factors are necessary for the activation of respiratory burst in phagocytes.¹⁴⁾ The detailed mechanism of active oxygen production *via* calcium-independent pathway in Con A-stimulated phagocytes is still unclear. However, it has been reported that elevated diacylglycerol without the activation of phosphatidylinositol-4,5-bis phosphate-specific phospholipase C is an important event associated with Con A-induced active oxygen generation by phagocytes other than the elevation of intracellular Ca^{2+} .¹⁵⁾ Since diacylglycerol is known to act as an inner activator of PKC,¹⁶⁾ diacylglycerol level in Con A-stimulated AMs may increase, and the augmentation of active oxygen generation by AMs may be caused by the rise in PKC activity based on the increase of diacylglycerol level by vitamin E.

Lastly, as our present data suggest that vitamin E can modulate the important function of active oxygen production by AMs, it is possible that the vitamin acts as an useful modulator of the AM immune functions.

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