Vitamin E at Physiological Levels Enhances Mouse Macrophages to Bind and Incorporate Oxidized Low Density Lipoprotein

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Edited by Y. Yamamoto, Univ. Tokyo, and accepted January 15, 2002 (received for review November 29, 2001)

Abstract: We have reported that high exogenous vitamin E (0.1-1 mM) inhibits thioglycollate-induced mouse peritoneal macrophages from binding and uptake of oxidized low density lipoprotein (oxLDL). However, in the present study, we found the amount of oxLDL bound to mouse macrophages increased with increasing levels of endogenous vitamin E in macrophages. Different vitamin E levels in macrophages were obtained by feeding mice with vitamin E deficient-, adequate- or rich-diet for 3 weeks; 0.02, 0.12 and 0.14 μg/mg protein, respectively. Exogenous vitamin E dose-dependently (up to 1 μM) increased the amount of oxLDL bound to macrophages from mice fed vitamin E adequate-diet and the numbers of lipid droplet positive macrophages. An increase of oxLDL binding with increasing of exogenous vitamin E was also observed in macrophages from mice fed vitamin E deficient-diet. Above results suggest that vitamin E at physiological levels enhances macrophages to bind and incorporate oxLDL.


Key words: macrophage, oxidized low density lipoprotein, vitamin E

1 Introduction

Lipid-laden foam cells found in the artery wall in early atherosclerotic lesions are considered to be originated from the macrophages that suffered massive accumulation of cholesterol within the cells (1, 2). The findings that macrophages avidly bind and take up oxidized LDL (ox LDL) in vitro (2, 3), which results in cholesterol accumulation, and that oxLDL is present in the atherosclerotic lesions (4-6) have closed up the role of macrophage binding and uptake of oxLDL in atherogenesis (2, 3). Oxidation of LDL has been considered to be an initial process of the formation of foam cells, and vitamin E is found to protect LDL from lipid peroxidation as an antioxidant in in vitro oxidation (7-10). Supplementation of vitamin E brings about the increased vitamin E content in LDL, and the susceptibility of LDL rich in vitamin E to in vitro oxidation is lowered (11).

Several reports addressed to the inhibitory effects of vitamin E on macrophage functions. Vitamin E on macrophages attenuates the production of superoxide and PGE2 from macrophages through the inhibition of cellular signal transduction unrelated to the antioxidant property (12-15). Enrichment of macrophages with vitamin E suppresses cholesterol esterification induced by modified LDL (16-18). In our previous studies, it has been shown that exogenously added vitamin E at the high levels in vitro reduces the macrophage recognition and uptake of oxLDL (19). In animal studies, supplementation of vitamin E reduces the incidences of atherosclerosis (20). In epidemiological studies, it has been shown that uptake of vitamin E is inversely correlated with the incidence of coronary heart disease (21, 22).
It is known that vitamin E has another adverse effect showing enhancement of responsiveness of cellular immune functions including macrophages. Dietary vitamin E supplementation has been shown to enhance phagocytic ability of alveolar macrophages in rats (23). The aim of the present study was to find the adverse effect of vitamin E on macrophage binding and uptake of oxLDL. It was found that macrophage binding of oxLDL was increased as the endogenous vitamin E level of the cells increased, and that macrophage binding and uptake were increased as the exogenously added vitamin E increased at the physiological levels.

2 Materials and Methods

2.1 Materials

Hanks’ balanced salt solution and N-2-hydroxethylpiperazine-N’-2-ethane sulfonic acid (HEPES) were obtained from Nissui Pharmaceutical Company (Tokyo, Japan) and Dojindo Laboratories (Kumamoto, Japan), respectively. RPMI1640 medium was obtained from Gibco Laboratories (Grand Island, NY). Fetal calf serum (FCS) was obtained from Biowhittaker (Walkersville, MD). Fluid thioglycollate medium was obtained from Difco Laboratories (Detroit, MI). Mayer’s hematoxylin solution was obtained from Wako Pure Chemical Industries (Osaka, Japan). Standard DL-α-tocopherol was obtained from Tokyo Kasei Kogyo Company (Tokyo, Japan). Standard bovine serum albumin (BSA) (γ-globulin-free and fatty acid-free) was obtained from Sigma Chemical (St. Louis, MO).

2.2 Animals and Diets

The protocol of animal preparations for the present experiment was approved by the Ethics Committee of our institute. Seventy 6-wk-old male ddY mice weighing 24-28 g were used. Five animals were housed together in a stainless steel cage in a room of controlled temperature at 23 ± 1°C, humidity at 55 ± 5% and lighting at 12 h dark-daylight cycle. The animals were allowed free access to diet. The animals were fed vitamin E adequate-solid diet, Clea rodent diet CE-2 containing protein at 226 g/kg, energy at 3600 kcal/kg, fat at 62 g/kg, fiber at 49 g/kg and vitamin E at 0 × 10⁻³ g/kg prepared by Oriental Yeast Company (Tokyo, Japan) were solidified by mixing 450 g diet and 230 g water for use. Dried solid vitamin E rich-diet, Orietal Composition Type MF containing protein at 238 g/kg, energy at 3570 kcal/kg, fat at 51 g/kg, fiber at 32 g/kg and vitamin E at 910 × 10⁻³ g/kg vitamin E, prepared by Oriental Yeast Company was used. Animals after acclimatization were divided into fourteen groups of five animals each: five groups were fed vitamin E adequate-diet, five groups vitamin E deficient-diet, and four groups vitamin E rich-diet for 3 wk. Weights of mice after feeding were 38-46 g.

2.3 Determination of Vitamin E Levels in Blood and Macrophages

Vitamin E levels of mouse blood and macrophages were determined according to the method described elsewhere (24). To 1.0 mL of heparinized blood from a mouse 1.0 mL of 60 mg/mL pyrogallol solution in ethanol and 1.0 mL of ethanol were added. After heating the mixture at 70°C for 2 min, 0.2 mL of 600 mg/mL KOH solution was added, and the mixture was heated at 70°C for 30 min. Vitamin E was extracted by addition of 2.5 mL of water and 5.0 mL of n-hexane and subsequent centrifugation at 1500 × g for 5 min.

The thioglycollate-induced peritoneal exudate cells from 5 mice were combined and washed twice with Ca²⁺- and Mg²⁺-free Dulbecco’s phosphate-buffered saline (DPBS(-)) by centrifugation (80 × g, 10 min) at 4°C to obtain macrophage pellet. To the pellet 1.0 mL of 600 mg/mL KOH was added. To 1.0 mL of the solution 4.0 mL of 60 mg/mL pyrogallol solution and 4.0 mL ethanol were added, and the mixture was heated at 70°C for 30 min. Vitamin E was extracted by addition of 10.0 mL of water and 5.0 mL of n-hexane and subsequent centrifugation. Protein in the KOH solution of macrophage pellet was determined according to the method of Lowry et al. (25) using BSA as a standard.

The upper n-hexane phase (4.0 mL) was evaporated to dryness to be redissolved into 0.2 mL of methanol. High performance liquid chromatography (HPLC) was carried out by using an Hitachi 655 liquid chromatograph (Tokyo, Japan) equipped with an Inertsil ODS-2 column (4.6 mm i.d. × 250 mm) (GL Sciences Incorporation, Tokyo, Japan) by injection of 10 μL of the sample solution in methanol and the column was eluted.
with a mobile phase composed of methanol/water (98: 2, vol/vol) at a flow rate of 1.4 mL/min. A fluorescent peak was detected at 292/335 nm with a Shimadzu RF-535 fluorescence spectrometer (Osaka, Japan). The peak due to vitamin E appeared at a retention time of 17-19 min. The amount of vitamin E in the sample was estimated by comparing the peak area with those of the calibration curve of the standard dl-α-tocopherol. Vitamin E levels in blood and macrophages was expressed as per mL blood and per mg protein, respectively.

### 2.4 Macrophages and Monolayer

Macrophages were obtained from the peritoneal cavity of mice with prior injection of 2-3 mL of 30 mg/mL thioglycollate medium 4 d before harvesting. The thioglycollate-induced peritoneal exudate cells were washed twice with Hanks' balanced salt solution by centrifugation (80 × g, 10 min) at 4°C. The cells were then resuspended in RPMI-20 mM HEPES medium at 0.70 × 10^6 cells/mL, and 0.2 mL of the cell suspension (64 μg protein) was loaded onto a round coverglass (18-mm diameter, 0.15-0.18 mm thick). After incubation at 37°C for 1 h, nonadherent cells were removed by washing three times with DPBS(-), and adherent cell monolayer of the macrophages was further incubated at 37°C for 20 h with 0.2 mL of RPMI medium supplemented with 0.01% (v/v) heat-inactivated FCS in a CO₂ incubator to ensure cell spreading on the coverglass.

### 2.5 Erythrocytes

Mouse blood was collected by cardiac puncture using heparin as an anticoagulant, and erythrocytes were centrifuged, washed three times with DPBS(-) after removal ofuffy coats and plasma, and resuspended in isotonic saline to make 2.5% (v/v) cell suspension.

### 2.6 Isolation and Oxidation of LDL

LDL was isolated according to method of Hatch and Lee (26) with minor modifications as described previously (27) using fresh human plasma. The LDL fraction obtained was dialyzed against 10 mM sodium phosphate-buffered saline (pH 7.4) at 4°C, and subjected to Cu²⁺-catalyzed oxidation according to the method of Steinbrecher et al. (28). Briefly, the dialyzed LDL was diluted to 2.0 mg/mL (as protein) with the same buffer, and incubated with 5 μM CuSO₄ at 37°C for 4 h. The reaction was terminated by addition of 50 μM butylated hydroxytoluene. The mixture was dialyzed against isotonic saline through which nitrogen gas had been bubbled, and stored at 4°C under nitrogen gas. Degree of lipid peroxidation of oxLDL thus obtained was estimated to be 18 nmol phosphatidylcholine hydroperoxide/mg protein and 14 nmol thiobarbituric acid-reactive substances/mg protein (29), whereas that of native LDL was estimated to be 1 nmol phosphatidylcholine hydroperoxide/mg protein and 3 nmol thiobarbituric acid-reactive substances/mg protein. This preparation was used as oxLDL. LDL only treated with dibutylated hydroxytoluene, followed by dialysis against saline, was used as native LDL. The protein content of LDL was determined by the method of Lowry et al. (25) using BSA as a standard.

### 2.7 LDL-coated Erythrocytes

Coating erythrocytes with native LDL or oxLDL was performed according to the method described elsewhere (29). Erythrocytes were obtained from mice fed vitamin E deficient-diet. Briefly, 1 volume of a 2.5% (v/v) erythrocyte suspension in saline was mixed with 0.25 volume of a solution of native LDL or oxLDL in saline (50 μg/mL), and the mixture was incubated at 37°C for 15 min with occasional mixing. The mixture was diluted with an equal volume of ice-cold DPBS(-) and centrifuged at 4°C and 170 × g for 15 min. The cells were washed twice with the same buffer at 4°C by centrifugation, and resuspended in RPMI-20 mM HEPES medium to make a 2% cell suspension. The cell suspension was prepared on the day of use. About 2-3 ng of LDL protein were found to be adsorbed to 10⁶ erythrocytes (29).

### 2.8 Binding of LDL-coated Erythrocytes to Macrophages

Binding of LDL-coated erythrocytes to macrophages was done according to the established method described elsewhere (29, 30). The macrophage monolayer from mice fed vitamin E adequate-diet, vitamin E deficient-diet or vitamin E rich-diet was used after washing with DPBS(-). For investigation of the effect of exogenously added vitamin E, the monolayer was pretreated at 37°C for 24 h in a CO₂ incubator with 0.2 mL of RPMI-0.01% (v/v) heat-inactivated FCS which had been mixed with 1% (v/v) ethanol containing 0.01-100 μM vitamin E at the final concentration, and washed with DPBS(-). On the macrophage monolayer, 200 μL of
2% (v/v) LDL-coated erythrocyte suspension were loaded. After incubation at 37°C for 1 h in RPMI-HEPES in a CO2 incubator, nonadherent cells were removed by washing with DPBS(-). The cells were fixed by 12.5 mg/mL glutaraldehyde in DPBS(-). The number of macrophages binding 5 or more LDL-coated erythrocytes was scored for random fields of the coverglass under phase-contrast microscopy.

2.9 Foam Cell Formation

The macrophage monolayer from a mouse fed vitamin E adequate-diet was pretreated at 37°C for 24 h with 0.2 mL RPMI-0.01% (v/v) heat-inactivated FCS which had been mixed with 1% (v/v) ethanol containing 0.001-100 μM vitamin E at the final concentration, and washed with DPBS(-). The monolayer was incubated at 37°C for 24 h in RPMI-0.01% (v/v) heat-inactivated FCS containing native LDL or oxLDL at 100 μg protein/mL in a CO2 incubator. The cells were fixed for 30 min by addition of 0.5 mL of 100 mg/mL formalin and washed with water. Neutral lipid droplets within the cells were stained with oil red O for 30 min, after which nuclei were stained with Mayer’s hematoxylin solution for 10 min. After being washed with water, the cells on the coverglass were examined by light microscopy. The number of macrophages containing lipid droplets stained red was scored under microscopy for the random fields of the coverglass. At least 200 cells were counted, and the percentages of the positively stained cells were determined.

2.10 Statistical Analysis

Data were analyzed by the Student’s t-test.

3 Results

3.1 Endogenous Vitamin E Content in Macrophages of Mice Supplemented with Diets Containing Different Levels of Vitamin E

Male ddY mice were fed vitamin E deficient-, adequate- and rich-diet for 3 wk. Vitamin E deficient-diet contained vitamin E at 0 × 10⁻³ g/kg dried solid, vitamin E adequate-diet vitamin E at 70 × 10⁻³ g/kg dried solid, and vitamin E rich-diet vitamin E at 910 × 10⁻³ g/kg dried solid. Vitamin E level in vitamin E rich-diet was 13 times as high as the level of vitamin E adequate-diet. Vitamin E levels in blood and thioglycollate-induced peritoneal macrophages were determined (Table 1). Vitamin E levels in blood were increased as the vitamin E levels in the diet increased: the levels in blood from vitamin E adequate- and vitamin E rich-diet were 4- and 8-fold higher as compared with the level of vitamin E deficient-diet. Endogenous vitamin E levels in macrophages of vitamin E deficient-, adequate- and rich-diet groups were 0.02, 0.12 and 0.14 μg/mg protein, respectively. Endogenous vitamin E level in macrophages from vitamin E adequate- and vitamin E rich-diet was similar and 6- and 7-fold higher than that from vitamin E deficient-diet. Endogenous vitamin E levels in macrophages were saturable by the vitamin E rich-diet.

3.2 Binding of oxLDL to Macrophages through oxLDL-coated Erythrocytes

In the course of our studies a simple macrophage binding assay for LDL has been demonstrated (29, 30).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Vitamin E Levels in Blood and Macrophages Obtained from Mice Fed a Vitamin E Deficient-, Vitamin E Adequate- and Vitamin E Rich-Diet,</th>
</tr>
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<tbody>
<tr>
<td>Diets</td>
<td>In blood (μg/ml blood)</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Vitamin E deficient-</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td>Vitamin E adequate-</td>
<td>1.91 ± 0.20</td>
</tr>
<tr>
<td>Vitamin E rich-</td>
<td>3.60 ± 0.32</td>
</tr>
</tbody>
</table>

aValues are expressed as means ± SD of five mice.
bValues are expressed as means ± SD of five tubes, each containing thioglycollate-induced macrophage suspensions obtained from five mice.
Superscriptc indicates a significantly difference from vitamin E deficient-diet as measured by Student’s t-test at P < 0.01.
In this assay, human oxLDL was coated on mouse erythrocytes, 2-3 ng of LDL protein being adsorbed to 10⁶ erythrocytes (29). OxLDL-coated erythrocytes bound much better than native LDL-coated erythrocytes. The binding is well-correlated to that of ¹²⁵I-radio-labeled LDL binding, and inhibited by the polyanion ligands for scavenger receptor class A (type I and type II) (29). This method is simple because the binding of LDL-coated erythrocytes to macrophages is visible under phase-contrast microscopy. Usefulness of the assay method in the present experiment was confirmed in this study. Thus, human LDL treated with or without Cu²⁺ ion to obtain oxLDL and native LDL, which were coated on erythrocytes obtained from mice fed vitamin E deficient-diet. The binding of native LDL-coated and oxLDL-coated erythrocytes to monolayer of thioglycolate-induced peritoneal macrophages obtained from mice fed vitamin E adequate-diet is shown in Fig. 1. It was found that oxLDL-coated erythrocytes bound to macrophages much better than native LDL-coated erythrocytes. This method was used in the following experiments of macrophage binding for LDL.

3.3 Effect of Dietary Vitamin E on the Macrophage Binding to oxLDL-coated Erythrocytes

Binding of thioglycollate-induced peritoneal macrophages from mice fed vitamin E deficient-, adequate- and rich-diet to oxLDL-coated erythrocytes was examined (Table 2). OxLDL-coated erythrocytes were loaded on the macrophage monolayer, and the monolayer was incubated at 37°C for 1 h. The percentages of macrophages binding 5 or more oxLDL-coated erythrocytes scored under phase-contrast microscopy were increased as the endogenous vitamin E levels in macrophages increased. The percentage of macrophages from vitamin E deficient mice that bound oxLDL-coated erythrocytes was 29.9%, that from vitamin E adequate mice was 36.3%, and that from vitamin E rich mice was 46.5%. The percentage of macrophages binding oxLDL-coated erythrocytes from vitamin E rich mice was significantly higher than that from vitamin E deficient mice. The result indicates that endogenous vitamin E in macrophages increased the macrophage binding of oxLDL in a level dependent fashion.

3.4 Effect of Preincubation of Macrophages with Vitamin E on the Macrophage Binding of oxLDL-coated Erythrocytes

The monolayer of thioglycollate-induced macrophages from mice fed a vitamin E deficient- or vitamin E adequate-diet was preincubated at 37°C for 24 h in media containing vitamin E at 0-100 µM (Fig.

![Fig. 1](https://example.com/figure1.png)  
**Fig. 1** Microscopic Observation of Thioglycollate-Induced Macrophages Binding Native LDL-Coated Erythrocytes (A) and oxLDL-Coated Erythrocytes (B). Thioglycollate-induced peritoneal macrophages obtained from a mouse fed vitamin E adequate-diet were used. Erythrocytes obtained from a mouse fed vitamin E deficient-diet were used. Native LDL- or oxLDL-coated erythrocytes was loaded on the macrophage monolayer, and the monolayer was incubated at 37°C for 1 h in RPMI-HEPES. Macrophages binding LDL-coated erythrocytes were observed under phase-contrast microscopy. Arrowheads show macrophages binding LDL-coated erythrocytes.
Table 2  Effect of Dietary Vitamin E Supplementation on the Macrophage Binding of OxLDL-Coated Erythrocytes.

<table>
<thead>
<tr>
<th>Macrophages</th>
<th>Macrophages binding oxLDL-coated erythrocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E deficient-</td>
<td>29.9 ± 4.1</td>
</tr>
<tr>
<td>Vitamin E adequate-</td>
<td>36.3 ± 7.1</td>
</tr>
<tr>
<td>Vitamin E rich-</td>
<td>46.5 ± 2.0</td>
</tr>
</tbody>
</table>

*Thioglycollate-induced macrophages were obtained from mice fed vitamin E deficient-, vitamin E adequate- and vitamin E rich-diet. OxLDL-coated erythrocytes were loaded on the macrophage monolayer, and the monolayer was incubated at 37°C for 1 h. The number of macrophages binding 5 or more oxLDL-coated erythrocytes was scored under phase-contrast microscopy. Results are expressed as means ± SD of five mice. Superscript* indicates a significantly different from vitamin E deficient-macrophages as measured by Student’s t-test at P < 0.01.

2) The levels of endogenous vitamin E in macrophages from vitamin E deficient and vitamin E adequate mice corresponded to 0.015 and 0.089 μM in the media of the exogenously added vitamin E, respectively. Native LDL- or oxLDL-coated erythrocytes were loaded on the monolayer, and the monolayer was incubated at 37°C for 1 h. The percentages of macrophages from vitamin E deficient mice and vitamin E adequate mice that were binding 5 or more native LDL-coated erythrocytes were low enough. The percentages of macrophages from vitamin E deficient mice binding oxLDL-coated erythrocytes sharply increased in a dose dependent fashion of the exogenously added vitamin E up to 10 μM. The percentages of macrophages from vitamin E adequate mice significantly increased but the increase was more gradual than that from vitamin E deficient mice up to 1 μM. The increased binding was significantly lowered at above 10 μM of exogenously added vitamin E, which is consistent with the previous observation showing that exogenously added vitamin E at more than 10 μM suppressed the binding of oxLDL to macrophages (19). The result indicates that exogenously added vitamin E at the low level ranges augmented the macrophage binding of oxLDL in a dose dependent fashion.

3.5 Effect of Preincubation of Macrophages with Vitamin E on the Formation of Foam Cells Induced by oxLDL

The monolayer of thioglycollate-induced macrophages from vitamin E adequate mice was preincubated at 37°C for 24 h in media containing vitamin E at 0-100 μM (Fig. 3). Native LDL or oxLDL was loaded on the monolayer, and the monolayer was incubated at 37°C for 24 h. The intracellular lipid droplets were stained with oil red O. The percentage of macrophages with lipid droplet induced by native LDL was low enough. The percentages of macrophages with lipid droplet induced by oxLDL increased in a dose dependent fashion of the exogenously added vitamin E. The percentage of macrophages with lipid droplet at 1 μM vitamin E was about 2-fold higher than that obtained without the exogenously added vitamin E. However, the augmented foam cell formation was decreased at the higher exogenously added vitamin E dose at more than 10 μM, which is consistent with the previous observation showing that exogenously added vitamin E at the high concentration suppressed the foam cell formation (19). The result indicates that exogenously added vitamin E at the low level ranges augmented the foam cell formation induced by oxLDL.

4 Discussion

Vitamin E level in mouse blood was enriched by dietary supplementation of vitamin E, but the enrichment was not linearly correlated with the amount of vitamin E in the diet. Vitamin E level in thioglycollate-induced peritoneal mouse macrophages was also enriched by dietary supplementation of vitamin E. Vitamin E level in macrophages of vitamin E rich mice was 7-fold higher than that in macrophage of vitamin E deficient mice. Enrichment of vitamin E in blood and macrophages by dietary supplementation may be sat-
urable process. The results are consistent with the earlier observation showing that plasma vitamin E enrichment is saturable process and plasma vitamin E levels on supplementation cease to increase (31, 32).

In our previous study, it has been shown that exogenously added vitamin E inhibits the macrophage binding and uptake of oxLDL when added to macrophages at the higher level ranges more than 10 μM (19). In the present study, it was found that the macrophage scavenging function for oxLDL was augmented as the endogenous vitamin E levels increased and the exogenously added vitamin E levels increased at the lower level ranges less than 10 or 1 μM. Scavenger receptor activity of macrophages (33, 34) for oxLDL may be augmented by endogenous vitamin E and exogenously added vitamin E at the physiological levels. Hence, vitamin E has biphasic adverse effects on the macrophage binding and uptake of oxLDL; enhancing effect at the physiological levels, and preventing effect at the higher levels.

Two mechanisms have addressed to the inhibition of the function of macrophages by vitamin E. One is an interference by vitamin E of macrophage cellular signal transduction. Superoxide production stimulated through the interrelated pathways of Ca²⁺ mobilization and activation of protein kinase C (12, 13) is inhibited by vitamin E on rat macrophages (14). Moreover, PGE₂ production and phospholipase A₂ activity in
Fig. 3 Effect of Preincubation of Macrophages with the Medium Containing Vitamin E on the Formation of Macrophage Foam Cells Induced by oxLDL. The monolayer of thioglycollate-induced macrophages from mice fed vitamin E adequate-diet was preincubated at 37°C for 24 h in RPMI-0.01% FCS containing vitamin E at the indicated final concentration. Native LDL (open circle) or oxLDL (100 μg protein/ml) (closed circle) was loaded on the monolayer, and the monolayer was incubated at 37°C for 24 h in RPMI-0.01% FCS. The intracellular lipid droplets were stained with oil red O. Control experiments without exogenously added vitamin E showed that 43.0% of macrophages were changed into lipid droplet positive cells. The data are expressed as mean percentages ± SD against the control in five determinations. Superscripts indicate significantly differences from the control and the preincubation of macrophages in the medium with 0.1 μM vitamin E as measured by Student's t-test at P < 0.01.

Macrophages from vitamin E-treated rats is significantly suppressed (15). Vitamin E on macrophages attenuates the production of superoxide and PGE₂ from macrophages probably through the inhibition of cellular signal transduction. Another is the effect of vitamin E on the cholesterol metabolism. Enrichment of macrophage-like J774 cells with vitamin E inhibits the macrophage mediated oxidation of LDL and inhibits cholesteryl ester formation in the cells (16). Enrichment of P388D macrophages with vitamin E reduces the cellular ratio of cholesteryl ester/free cholesterol without changing cholesteryl ester accumulation induced by modified LDL (17). Enrichment of J774 cells with vitamin E decreased the acetylated LDL-induced cholesteryl ester formation in the cells, due to the suppression of acyl CoA: cholesterol acyltransferase, indicating that vitamin E reduces the uptake of modified LDL and suppresses the enzyme activity (18).

On the other hand, vitamin E has another adverse effect unrelated to its antioxidant property showing enhancement of responsiveness of cellular immune functions. Decrease in antigen expression is observed in vitamin E deficient rats (35). Dietary vitamin E supplementation has been shown to enhance phagocytic ability of alveolar macrophages in rats (23). The in vitro addition of vitamin E increases concanavalin A-stimulated cell proliferation and IL-2 production when macrophages from mice were co-cultured with purified T cells (36, 37).

The previous study (19) and the present study on the effects of vitamin E on macrophages showed that vitamin E has biphasic adverse effects on the macrophage binding and uptake of oxLDL, enhancing and inhibiting effects depending on the levels of the vitamin E. The enhancing effect of vitamin E may be due to vitamin E responsiveness of cellular immune functions of macrophages to bind and take up oxLDL. The inhibiting effect of vitamin E against the macrophage activity to bind and take up oxLDL may be due to retardation of the cellular signal transduction and/or cholesterol metabolism.

Acknowledgment
This work was supported in part by a Grant for Private Universities provided by Japan Private School Promotion Foundation.

References


