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# Review Article

# Vitamin E and Leukocyte–Endothelial Cell Interactions

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## ABSTRACT

**Leukocyte–endothelial cell interactions are mediated by adhesion molecules, expression of which is modulated by cytokines and chemical mediators in the early phase of inflammatory and immunologic reactions, including the development of atherosclerosis. Vitamin E is a lipid-soluble antioxidant that is present in all cell membranes at a low concentration and is reported to be an anti-atherogenic agent. Recently, it was reported that vitamin E inhibits the activation of protein kinase C and nuclear factor-kappa B (NF-κB). We demonstrated that vitamin E can prevent leukocyte–endothelial cell adhesion by inhibiting signal transduction involved in the surface expression of adhesion molecules by leukocytes and endothelial cells. These results suggest that vitamin E may have a protective effect against the progression of inflammation and atherosclerosis.** Antiox. Redox Signal. 2, 821–825.

## ADHESION MOLECULES INVOLVED IN LEUKOCYTE-ENDOTHELIAL CELL INTERACTIONS

ADHERENCE OF POLYMORPHONUCLEAR LEUKOCYTES (PMN) to the endothelium and migration of PMN into the extravascular space are characteristic steps in the process of inflammation. PMN–endothelial cell interactions are known to be mediated by adhesion molecules that are expressed on both types of cells, and the expression of these molecules is modulated by inflammatory cytokines and lipid mediators (Springer, 1990). Among the adhesion molecules expressed on leukocytes, L-selectin mediates rolling of leukocytes along the endothelium, whereas the CD11/CD18 integrin family, which is comprised of an immunologically distinct $\alpha$-subunit (CD11a, CD11b, or CD11c) and a common $\beta_2$-subunit (CD18), plays a major role in the adhesion and transendothelial migration of PMN both *in vitro* and *in vivo* (Tonnesen, 1989; Carlos and Harlan, 1990). Chemotactic factor-induced adherence of PMN is mainly dependent on CD11b/CD18. Up-regulation of CD11b/CD18 has been reported to occur within a few minutes and involves receptor rearrangement or the translocation of preformed receptors from intracellular stores, similar to the up-regulation of P-selectin expression on endothelial cells (EC) (Zimmerman *et al.*, 1992; Granger and Kubes, 1994). In addition, the process of leukocyte adherence to EC involves increased surface expression of membrane glycoproteins, E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1) on EC, which requires several hours to reach a maximal level after endothelial cell activation. After up-regulation, these molecules on EC interact with receptors on leukocytes. Specifically, E-selectin, ICAM-1, and VCAM-1 on EC interact with sialyl Lewis-X-containing molecules, CD11b/CD18, and VLA-4 integrins ($\alpha_4\beta_1$), respectively,

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on leukocytes (Springer, 1990; Zimmerman *et al.*, 1992; Granger and Kubes, 1994).

## VITAMIN E AND NEUTROPHIL-DEPENDENT ADHESION TO ENDOTHELIAL CELLS

Oxidized low-density lipoprotein (oxLDL) is an important indicator of oxidative stress as well as being a potent chemotactic agent that is involved in the infiltration of leukocytes during the early processes of atherosclerosis (Steinberg *et al.*, 1989; Lehr *et al.*, 1993). OxLDL exhibits a wide variety of potentially atherogenic properties *in vitro*, including stimulation of monocyte migration (Navab *et al.*, 1991), promotion of neutrophil and mononuclear cell adhesion to EC (Quinn *et al.*, 1987; Fröstegard *et al.*, 1991; Couffinhal *et al.*, 1993), and inhibition of endothelium-dependent vasodilation (Simon *et al.*, 1990), but there have been few reports that oxLDL induces integrin up-regulation. In a recent study (Yoshida *et al.*, 1999), we showed that oxLDL, but not native LDL, stimulates the up-regulation of CD11b/CD18 expression on PMN. We also found that lysophosphatidylcholine (lysoPC), a potent component of oxLDL and an inducer of transient ICAM-1 and VCAM-1 expression by human aortic EC (Kume *et al.*, 1992), did not induce the up-regulation of CD11b/CD18. These results suggested that a component of oxLDL other than lysoPC played a role in the up-regulation of CD11b/CD18 expression on PMN.

$\alpha$-Tocopherol ($\alpha$-Toc) is a lipid-soluble vitamin and a chain-breaking tissue antioxidant that is present in all cell membranes at a low concentration and is reported to be an antiatherogenic agent (Burton and Ingold, 1989; Janero, 1991). $\alpha$-Toc is also thought to be an immunemodulator, enhancing both cell-mediated and humoral immunity (Tengerdy, 1989), although the mechanism of this action has not been determined. The cytoprotective effect of $\alpha$-Toc is attributed to its ability to act as a scavenger of highly reactive oxygen radicals, thus stabilizing membranes against lipid peroxidation (Chow, 1991; Urano and Matsuo, 1989), and to its inhibition of protein kinase C (PKC), as shown by studies using rat brain homogenates (Mahoney and Azzi, 1988), permeabilized vascular smooth muscle cells, and neuroblastomas (Boscoboinik *et al.*, 1991). Recent studies have suggested that vitamin E supplementation may be of benefit in the prevention of coronary artery disease (Rimm *et al.*, 1993; Stampfer *et al.*, 1993; Stephens *et al.*, 1996). It has also been suggested that $\alpha$-Toc may act at the level of vascular cell gene expression (Faruqi *et al.*, 1994).

The effects of antioxidants on leukocyte–endothelial cell interactions involved in atherosclerosis and heart diseases have attracted considerable attention. Recently, we found that vitamin E decreased the level of CD11b/CD18 expression on human PMN monocytes induced by oxLDL, platelet-activating factor (PAF), or *N*-formyl methionyl-leucyl-phenylalanine (fMLP) (Yoshikawa *et al.*, 1998; Yoshida *et al.*, 1999; Terasawa *et al.*, 2000). An inhibitory effect of vitamin E on CD11/CD18 expression was also demonstrated in an *ex vivo* study. Using blood samples obtained from volunteers who received $\alpha$-Tox (600 mg/day) for 10 days, it was shown that CD11b/CD18 expression induced by fMLP or oxLDL was significantly reduced after administration of vitamin E. Also the level of CD11b/CD18 expression was inversely correlated with the serum vitamin E concentration. Furthermore, adhesion of fMLP- or oxLDL-stimulated PMN to intact EC was suppressed by $\alpha$-Toc (50–200 $\mu M$) in a concentration-dependent manner.

On the other hand, vitamin E did not inhibit CD11b/CD18 up-regulation and PMN adherence induced by phorbol 12-myristate-13-acetate (PMA), a direct activator of PKC, whereas a PKC inhibitor suppressed the up-regulation of CD11b/CD18 by fMLP, oxLDL, or PMA in a concentration-dependent manner. Thus, fMLP-, oxLDL-, and PMA-induced up-regulation of CD11b/CD18 occurs via direct or indirect activation of PKC. Although $\alpha$-Toc did not affect PKC activity in unstimulated cells, it slightly inhibited the increase of PKC activity in response to fMLP or oxLDL stimulation, but not that caused by PMA stimulation, suggesting that $\alpha$-Toc tends to reduce the response of PKC to indirect stimuli. It is probable that $\alpha$-Toc acts on signal transduction pathways from surface receptors to PKC activation. It has been

reported that a decrease in the membrane fluidity of stimulated leukocytes can induce up-regulation of CD11b/CD18 via modulation of receptor translocation from intracellular stores (Detmers *et al.*, 1987; Vosatka *et al.*, 1988). α-Toc may stabilize membrane fluidity because of its ability to act as a scavenger of reactive oxygen radicals, thus protecting membranes against lipid peroxidation (Urano and Matsuo, 1989; Chow, 1991). Further studies are needed to determine the molecular mechanisms by which α-Toc inhibits up-regulation of CD11b/CD18 on activated PMN.

Neutrophil activation also triggers the functional activation of preexisting cell-surface CD11b/CD18, presumably through conformational and/or topological alternations (Buyon *et al.*, 1988). Neutrophil adhesion may also be attributed to the phosphorylated activation as well as up-regulation of CD11b/CD18 (Schleiffenbaum *et al.*, 1989). Therefore, one possibility is that α-Toc may prevent neutrophil adhesion by inhibiting the functional activation of constitutively expressed CD18.

In summary, the present study suggests that α-Toc can block the adhesion of PMN to EC stimulated by fMLP or oxLDL, probably by modulating quantitative and/or functional changes of CD11b/CD18. Accordingly, α-Toc may have a protective effect against the progression of inflammation and atherosclerosis by modulating leukocyte-dependent interactions with EC.

## VITAMIN E AND ENDOTHELIAL–DEPENDENT LEUKOCYTE ADHESION

Endothelial adhesion molecules play an important role in the rolling and adhesion of leukocytes within the circulatory system. It is well known that several cytokines, such as interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α), induce the surface expression of ICAM-1 and VCAM-1 on endothelial cells through the activation of transcription factor, nuclear factor-kappa B (NF-κB), followed by synthesis of its mRNA (Iademarco *et al.*, 1992; Hou *et al.*, 1994). Recently, LDL and oxLDL have been shown to induce the up-regulation of adhesion molecules on endothelial cells. In our experiments, ICAM-1 and VCAM-1 expression on oxLDL-stimulated human umbilical vein endothelial cells (HUVEC) was found to occur in the same fashion as after stimulation with IL-1 (Yoshida *et al.*, 1997; Yoshikawa *et al.*, 1998). However, LDL did not have any effect on the expression of endothelial adhesion molecules. These results suggest that oxLDL may induce almost the same signal transduction pathway for the synthesis of ICAM-1 and VCAM-1 as that stimulated by IL-1 (Yoshikawa *et al.*, 1998), but the precise mechanisms involved still need to be investigated. In addition, we found that vitamin E inhibited the synthesis of mRNA and protein for ICAM-1 and VCAM-1 by HUVEC in response to stimulation with oxLDL or IL-1 (Yoshikawa *et al.*, 1998). Previously, we showed that vitamin E inhibited the surface expression of ICAM-1 and VCAM-1 on EC exposed to IL-1 (Yoshida *et al.*, 1999). Cominacini *et al.* (1997) also reported that vitamin E and probucol decreased ICAM-1 and VCAM-1 expression on HUVEC in response to oxLDL stimulation. Recently, it has been suggested that active oxygen species are implicated in the activation of NF-κB, which is necessary for the expression of various adhesion molecules. Nakamura *et al.* (1998) and Erl *et al.* (1997) have reported that some forms of vitamin E inhibit NF-κB activation. These findings suggest that vitamin E may prevent NF-κB-

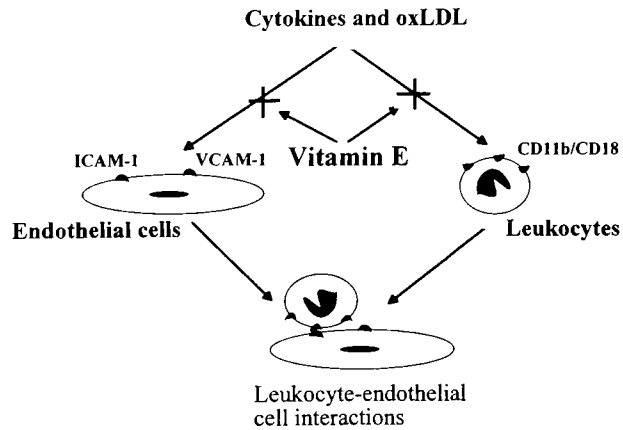


**FIG. 1. Scheme of inhibitory effect of vitamin E on leukocyte–endothelial cell interactions.** Cytokines and oxLDL induce surface expression of ICAM-1 and VCAM-1 on endothelial cells and CD11b/CD18 on leukocytes. Vitamin E inhibits leukocyte–endothelial cell adhesion by reducing up-regulation of adhesion molecules.

mediated expression of adhesion molecule mRNAs and proteins by reducing oxidative stress on EC.

In summary, the results summarized here indicate that vitamin E can reduce endothelial-dependent leukocyte adhesion by inhibiting signal transduction involved in the surface expression of ICAM-1 and VCAM-1 on EC, suggesting that vitamin E may act as an anti-inflammatory and anti-atherogenic agent (Fig. 1).

## ABBREVIATIONS

fMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; ICAM-1, intercellular adhesion molecule-1; NF-κB, nuclear factor-kappa B; oxLDL, oxidized low density lipoprotein; PAF, platelet activating factor; PKC, protein kinase C; PMA, phorbor 12-myristate 13-acetate; PMN, polymorphonuclear leukocytes; α-Toc, α-Tocopherol; VCAM-1, vascular adhesion molecule-1.

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