Age-associated increase in PGE$_2$ synthesis and COX activity in murine macrophages is reversed by vitamin E

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Age-associated increase in PGE$_2$ synthesis and COX activity in murine macrophages is reversed by vitamin E. Am. J. Physiol. 275 (Cell Physiol. 44): C661–C668, 1998.—We previously showed that increased macrophage PGE$_2$ production with age is due to enhanced cyclooxygenase (COX) activity and COX-2 expression. This study determined the effect of vitamin E supplementation on macrophage PGE$_2$ synthesis in young and old mice and its underlying mechanism. Mice were fed 30 or 500 parts per million vitamin E for 30 days. Lipopolysaccharide (LPS)-stimulated macrophages from old mice produced significantly more PGE$_2$ than those from young mice. Vitamin E supplementation reversed the increased PGE$_2$ production in old mice but had no effect on macrophage PGE$_2$ production in young mice. In both LPS-stimulated and unstimulated macrophages, COX activity was significantly higher in old than in young mice at all intervals. Vitamin E supplementation completely reversed the increased COX activity in old mice to levels comparable to those of young mice but had no effect on macrophage COX activity of young mice or on COX-1 and COX-2 protein or COX-2 mRNA expression in young or old mice. Thus vitamin E reverses the age-associated increase in macrophage PGE$_2$ production and COX activity. Vitamin E exerts its effect posttranslationally, by inhibiting COX activity.

AGING IS ASSOCIATED WITH depressed immune function, and decline in immunologic vigor contributes to many age-associated infections and neoplastic diseases as well as to the prolonged postillness recovery often observed in the elderly. The age-associated changes are mostly observed in T cell-mediated functions, which are believed to be due to intrinsic changes in T cells and increased production of suppressive factors from macrophages (4, 15, 30). We, as well as others, have shown that macrophages and splenocytes from old mice and peripheral blood mononuclear cells from elderly human subjects synthesize significantly more PGE$_2$ (a compound with inhibitory effects on T cell-mediated function) than their young counterparts (2, 4, 15, 26, 28, 36). Furthermore, we showed that increased PGE$_2$ production contributes to the age-associated decline in T cell proliferation and interleukin (IL)-2 production (4). The increase in cyclooxygenase (COX) products has also been observed in other tissues (29, 43, 45). We recently showed that the age-associated increase in lipopolysaccharide (LPS)-stimulated PGE$_2$ production is due to an increase in COX activity, which is, in turn, mainly due to increased expression of COX-2 (16).

COX is the rate-limiting synthase in the biosynthesis of prostaglandins and has bifunctional catalytic properties. It catalyzes both formation of PGG$_2$ from arachidonic acid via its COX activity and subsequent reduction of PGG$_2$ to PGL$_2$ via its peroxidase activity (8). Many studies have shown that at least two forms of the enzyme COX exist, a constitutive form (COX-1) and an induced form (COX-2) (19, 31). The two enzymes appear to be functionally distinct, having different distributions and responsiveness patterns to different stimuli. COX-1 is constitutively expressed, whereas COX-2 is regulated by growth factors, tumor promoters, cytokines, and mitogens such as LPS and glucocorticoids (13, 42).

Prostaglandins are potent mediators of intercellular communication and as such modulate a variety of normal cellular responses as well as mediate some pathophysiologic processes. At low concentrations PGE$_2$ is believed to be necessary for certain aspects of cellular immunity, whereas at higher concentrations PGE$_2$ has been reported to be immunosuppressive for the T cell-mediated response, as demonstrated by decreased T cell proliferation and IL-2 production (11, 12). PGE$_2$ has also been shown to inhibit immune response-related signals such as phosphatidylinositol turnover, phytomagglutinin- and anti-CD3-stimulated increases in Ca$^{2+}$, and tyrosine phosphorylation of a 100-kDa protein, which increases following mitogenic stimulation (1, 5, 33). PGE$_2$ has been implicated in maintaining the balance between T helper 1 and 2 cells as well (34).

We have previously reported that vitamin E supplementation significantly enhanced T cell proliferation, IL-2 production, and delayed-type hypersensitivity skin response in aged mice and humans (26, 28). In a recent study, we showed that vitamin E supplementation of healthy elderly subjects improved their in vivo indexes of cell-mediated immune function, including response to vaccine, while reducing the incidence of self-reported infections (27). We further showed (4) that vitamin E exerts its immunoenhancing effect in aged mice mainly...
through inhibition of macrophage PGE2-mediated suppression.

The mechanism of the vitamin E-induced decrease in macrophage PGE2 production is not known. The purposes of the present study were, therefore, 1) to determine the effect of vitamin E on macrophage PGE2 production of young and old mice and 2) to determine whether the vitamin E-induced change in PGE2 production is due to its effect on expression of COX-2. We report here that vitamin E supplementation decreases PGE2 production and COX activity in macrophages from old but not young animals. This effect of vitamin E is not mediated through reduction in COX-1 or COX-2 expression; rather, vitamin E exerts its effect posttranslationally by affecting COX activity.

MATERIALS AND METHODS

Animals. Specific pathogen-free male young (6 mo) and old (24 mo) C57BL/6NA mice were obtained from National Institute on Aging colonies at Charles River Laboratories (Kingston, NY). The mean life span of these mice is 28 mo. Forty young and forty old mice were used to determine PGE2 production and COX activity, and ninety-six old mice were used to determine the mRNA and protein expression in four experiments. Mice were housed singly in microisolator cages at constant temperature (23°C), with a 12:12-h light-dark cycle. Mice were daily fed semisynthetic diets (Table 1) containing 30 (adequate level) or 500 parts per million (ppm) vitamin E for 30 days. The leftover diet from the previous day was discarded. Mice were paired by age and euthanized via CO2 asphyxiation. All conditions and handling of the animals were approved by the Animal Care and Use Committee of the Jean Mayer Human Nutrition Research Center on Aging at Tufts University and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice exhibiting visible tumors or splenomegaly were excluded from the study.

Peritoneal macrophage isolation. Peritoneal exudate cells were obtained by peritoneal lavage with cold Ca2+- and Mg2+-free Hanks’ balanced salt solution (GIBCO, Grand Island, NY). Cells from 2 mice were pooled for measurement of PGE2 production and COX activity (final n = 10), and cells from 12 old mice per treatment group were pooled for analysis of COX mRNA and protein levels (final n = 4). Peritoneal exudate cells were enriched for macrophages using the method of Kumagai et al. (22). Briefly, peritoneal macrophages were suspended in endotoxin-free RPMI 1640 (ETRPMI; Bio Whitaker, Walkersville, MD) medium supplemented with 25 mM HEPES, 2 mM glutamine (GIBCO), 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO), and 5% FCS. The cells were plated on six-well plates (Falcon Labware, Lincoln Park, NJ) that had been previously blocked with FCS and allowed to adhere for 2 h at 37°C in 5% CO2, at which time nonadherent cells were removed by vigorous washing. Peritoneal macrophages prepared in this manner were at least 90% macrophages, as assessed by Mac-1 and F4/80 cell surface antibody.

Table 1. Composition of basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight, %</th>
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<tbody>
<tr>
<td>Vitamin-free casein*</td>
<td>18.00</td>
</tr>
<tr>
<td>Corn starch*</td>
<td>33.55</td>
</tr>
<tr>
<td>Sucrose*</td>
<td>33.40</td>
</tr>
<tr>
<td>Cellulose†</td>
<td>5.00</td>
</tr>
<tr>
<td>dl-Methioninet</td>
<td>0.30</td>
</tr>
<tr>
<td>Choline bitartrate†</td>
<td>0.25</td>
</tr>
<tr>
<td>AIN 76A salt mix</td>
<td>3.50</td>
</tr>
<tr>
<td>AIN 76A vitamin mix†</td>
<td>1.00</td>
</tr>
<tr>
<td>Lard (tocopherol stripped)†</td>
<td>3.80</td>
</tr>
<tr>
<td>Corn oil (tocopherol stripped)†</td>
<td>1.20</td>
</tr>
</tbody>
</table>

*Dysts (Bethlehem, PA). †Harlan Teklad (Madison, WI). ‡Bio-Serv (Frenchtown, NJ).

The percentage of macrophages that adhered to the plates did not differ among the different age and diet groups (data not shown).

PGE2 production and COX enzyme activity. Peritoneal macrophages were isolated, pooled, and plated (5 × 10^5 cells/well) as described previously. One milliliter of ETRPMI containing 5% FCS was incubated with either 0 or 5 μg/ml LPS (Sigma, St. Louis, MO) was added to each well. Plates were incubated at 37°C in 5% CO2 for 0, 6, 12, and 24 h, at which time the supernatant was removed and immediately frozen and stored at −70°C for analysis of ex vivo production of PGE2. The time course study was not conducted beyond 24 h because our previous study showed no further increase in PGE2 production between 24 and 48 h (16). After removal of supernatants, cells were layered with 1 ml of ETRPMI medium containing 30 μM arachidonic acid and incubated at 37°C for 10 min for determination of COX activity as described by Fu et al. (10).

Total cellular COX activity can be measured by adding excess exogenous arachidonic acid to macrophages, because the intracellular enzyme pool is saturated with the substrate and is functioning at maximal velocity. After 10 min, COX enzyme activity was inhibited with 2.1 mM aspirin. Supernatants were immediately removed and stored at −70°C. Cells were then incubated with 1 M NaOH for 5 min, at which time the supernatant was removed and stored at −20°C for protein analysis by Bio-Rad protein assay reagent (Bio-Rad, Richmond, CA). PGE2 was measured by RIA as previously described (15).

COX mRNA measurement. A fragment of COX-1, from bp 1091 to bp 1291, and a fragment of COX-2, from bp 1590 to bp 1791, were amplified by PCR, blunted, and subcloned into the Xho site of PBS IIKS (Stratagene, La Jolla, CA). Pvu II was used to linearize both COX-1 and COX-2 templates for labeling. A pTRI-Actin-Mouse (Ambion, Austin, TX), linearized with Hind III, was used as an internal control.

On the basis of results of previous time course experiments (16), in this study, peritoneal macrophages were stimulated for 1, 2, 4, and 8 h with 5 μg/ml LPS in ETRPMI in the presence of 5% FCS. Total RNA was prepared by extraction with the TRIzol reagent (GIBCO BRL, Gaithersburg, MD). The labeled RNA probe was hybridized with known amounts of total RNA and treated with ribonuclease. The sizes of the protected fragments analyzed by electrophoresis were 210 bp for COX-1, 311 bp for COX-2, and 249 bp for actin. The signals were quantified by scanning densitometry using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The ratios of the densitometer readings of the individual samples relative to actin were calculated to determine the level of COX-1 and COX-2 expression at the different time points. An RS-2 cell line (gift of Dr. Daniel Simmons, Brigham Young University, Provo, UT) was used as the positive control for COX-2, and tRNA was used as the negative control.

Protein measurement: Western blot. On the basis of previous results (16), peritoneal macrophages were stimulated for 0, 6, 12, and 24 h with 5 μg/ml LPS in ETRPMI in the presence of 5% FCS. After stimulation, macrophages from young and old animals were homogenized in lysis buffer [50 mM Tris, 10 mM EDTA, 1% Tween 20, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 10 μM leupeptin (pH 7.4)]. Protein concentration was determined using the bichinchoninic acid

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protein assay (Sigma). Samples of 40 or 60 µg of lysate protein were electrophoresed on a 7.5% SDS-polyacrylamide slab gel and transblotted to nitrocellulose membrane (Bio-Rad). COX-2 and COX-1 protein levels were determined by using rabbit anti-COX-1 and rabbit anti-COX-2 antibodies with standard detection techniques (ECL system, Amersham; Tropix, Bradford, MA). The specific COX-1 and COX-2 signals were quantified by scanning densitometry using Image Quant software. Because the level of COX-1 does not change with stimulation, the levels of COX-2 protein are reported as the ratios of the densitometer readings of COX-2 relative to COX-1. In addition, COX-2 levels were expressed relative to Coomassie blue stain protein. Results obtained with Coomassie blue stain were the same as when COX-2 was expressed relative to COX-1 (data not shown).

Results

Production of PGE₂. The production of PGE₂ from peritoneal macrophages without and with LPS stimulation is presented in Fig. 1, A and B, respectively. PGE₂ production in unstimulated cultures was low and did not show a significant change with time in macrophages from either young or old mice. There was no significant difference between young and old mice in PGE₂ levels at any of the time points tested. Vitamin E supplementation did not cause a significant change in COX activity but had no effect on PGE₂ production in either age group (Fig. 1A). In LPS-stimulated macrophages, PGE₂ production significantly increased with time in macrophages from both age groups. Macrophages from old mice fed the control diet showed significantly higher PGE₂ levels at 12 and 24 h compared with macrophages from young mice fed the control diet. Vitamin E supplementation completely eliminated this age-related increase in PGE₂ production, so that there was no significant difference in PGE₂ production between old mice fed 500 ppm vitamin E and young mice fed control or vitamin E-supplemented diets. Vitamin E supplementation, however, did not have a significant effect on PGE₂ production in young mice (Fig. 1B).

Fig. 1. Accumulated production of PGE₂ by peritoneal macrophages isolated from young and old mice. Young and old mice were fed diets containing either 30 or 500 parts per million (ppm) vitamin E (E 30 and E 500, respectively) for 30 days. Peritoneal macrophages were isolated and cultured (5 × 10⁵ cells/well) in absence (A) or presence (B) of 5 µg/ml lipopolysaccharide (LPS) for 0, 6, 12, or 24 h at 37°C. Supernatants were removed at appropriate time points and stored at −70°C. PGE₂ concentration was determined by RIA. Cells were lysed by 1 M NaOH, and total cell protein was measured using a Bio-Rad protein assay kit. Means = SE for n = 10 in each age and diet group.

* Significant difference at P < 0.05 between young and old mice fed 30 ppm vitamin E.

COX activity. COX activity in unstimulated and LPS-stimulated macrophages is presented in Fig. 2, A and B, respectively. In unstimulated cultures, COX activity did not change with time in either age group. Macrophages from old mice fed the control diet showed significantly higher COX activity than those from young mice fed the control diet at all time points. Vitamin E supplementation did not have a significant effect on COX activity in young mice; however, it significantly decreased COX activity in old mice, so that there was no significant difference in COX activity between old mice fed 500 ppm vitamin E and young mice fed control or vitamin E-supplemented diets (Fig. 2A). After macrophages were stimulated with LPS, COX activity increased with time in both age groups. Macrophages from old mice fed 30 ppm vitamin E demonstrated significantly higher COX activity than those from young mice at all time points, and again supplementation with 500 ppm vitamin E completely suppressed this age-related increase in COX activity but had no effect.
on COX activity of macrophages from young mice (Fig. 2B). Because LPS does not induce COX-1, the activity in LPS-stimulated cultures is mainly due to COX-2 (inducible form). The activity in unstimulated cultures may be due to either COX-1 or COX-2. The latter is more likely, since, under these conditions, COX-1 mRNA and protein are barely detectable (see Fig. 4A), whereas COX-2 mRNA and protein can be detected at low levels (see Fig. 4A). Serum (present in our cultures) has been shown to induce COX-2 (14). Furthermore, we have previously shown that the age-related increase in COX-2 activity is mainly due to increased expression of COX-2 and not COX-1 (16).

COX-2 mRNA and protein expression. Because we previously showed that the age-associated increase in PGE2 production is due to increased COX-2 expression (16), we investigated the possibility that vitamin E decreases PGE2 production by reducing transcription and/or translation of COX-2 gene. To test this hypothesis, we compared COX-2 mRNA and protein levels between macrophages from mice fed 30 or 500 ppm vitamin E. As previously reported, COX-2 gene was induced by LPS, and the mRNA reached maximum levels 2–4 h after stimulation (Fig. 3, A and B). Dietary treatment did not significantly affect the level of mRNA detected before (0 h) or after (2, 4, or 6 h) LPS stimulation. Furthermore, neither the level of COX-2 protein (Western blot; Fig. 4A) nor de novo synthesis of COX-2 (immunoprecipitation; data not shown) following LPS stimulation was affected by the level of vitamin E in the diet. In contrast to COX-2, COX-1 mRNA (data not shown) and protein were barely detectable in macrophages from either treatment group. Consistent with reports that COX-1 acts as a “housekeeping” gene, LPS stimulation and dietary treatment had no effect (P = 0.62) on its expression (Fig. 4B). These results demonstrate that vitamin E does not affect expression of either COX-1 or COX-2 genes but decreases COX activity by influencing posttranslational regulation.

DISCUSSION

In this study, we confirmed our previously reported findings that PGE2 production by mouse macrophages is increased with age and that this change is a consequence of age-associated upregulation of COX activity (16). Furthermore, we demonstrated that dietary supplementation with vitamin E reduces PGE2 production by macrophages from old mice mainly through the suppression of age-associated increase in COX activity and not substrate availability. The vitamin E-induced suppression of COX activity was not due to a decrease in expression of protein or mRNA for COX-1 or COX-2, thus indicating regulation by vitamin E at the posttranslational level.

In the present study, we investigated the effect of vitamin E on PGE2 production by macrophages from

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**Fig. 2.** Cyclooxygenase (COX) activity in peritoneal macrophages isolated from young and old mice. Dietary treatment and cell culture conditions were as for Fig. 1. After removal of culture supernatants for assay of accumulated PGE2 production at each time point, cells were layered with 1 ml of ETRPMI 1640 containing 30 µM arachidonic acid. After 10 min of incubation at 37°C, aspirin (2.1 mM) was added to stop reaction. Supernatants were then collected for PGE2 analysis, and cells were lysed for protein analysis as described for Fig. 1. COX activity (means ± SE for n = 10 in each age and diet group) is expressed as conversion of arachidonic acid to PGE2 (pg·µg protein⁻¹·10 min⁻¹). *Significant difference at P < 0.05 between young and old mice fed 30 ppm vitamin E.

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**Fig. 3.** COX-2 mRNA levels in macrophages from old C57BL/6NIA mice fed a semipurified diet containing 30 or 500 ppm vitamin E (means ± SE of 4 independent experiments). Macrophages were cultured in presence of 5 µg/ml LPS for specified times. RNase protection assay was used to assess mRNA levels. Autoradiographs were scanned, and each COX-2 signal was normalized to signal of its corresponding β-actin band.
young and old animals and examined the mechanism of vitamin E-induced inhibition of PGE$_2$ production. PGE$_2$ level is regulated by substrate availability, i.e., arachidonic acid level, and the activity of enzyme COX. The activity of the enzyme is determined by the level of the enzyme and requires hydroperoxide as an activator (17, 41). Because our previous study showed that the age-associated difference in PGE$_2$ production is not due to substrate availability but rather is a reflection of changes in COX activity (15, 16), we focused on COX activity in determining the mechanism of vitamin E-induced decrease of PGE$_2$ production in old mice. To show that the effect of vitamin E was due to decreased COX activity and not substrate availability, PGE$_2$ production was measured in the presence of excess exogenously added arachidonic acid. As can be seen in Fig. 2, vitamin E inhibited PGE$_2$ production when substrate was not limited. This is further supported by the fact that the inhibitory effect of vitamin E was age specific; no significant effect on LPS-stimulated PGE$_2$ production or COX activity was observed in young macrophages. However, vitamin E supplementation significantly decreased both LPS-stimulated PGE$_2$ production and COX activity in macrophages from old mice. It is interesting to note that, for up to 24 h of culture, vitamin E inhibited LPS-stimulated PGE$_2$ accumulation and COX activity to the same extent, i.e., 60%, further suggesting that vitamin E inhibition of PGE$_2$ production is mainly through its inhibition of COX activity rather than an effect on substrate release. To show that the effect of vitamin E is exerted on COX and not on the activity of the downstream enzyme PGE$_2$ isomerase, we investigated the effect of vitamin E on another COX product, thromboxane (TX) A$_2$ (measured as its stable hydrolytic product TXB$_2$), in the supernatant from COX activity cultures. The results show that vitamin E had no effect on the conversion of exogenous arachidonic acid to TXA$_2$ in LPS-stimulated macrophages of young mice (10.6 ± 1.8 and 8.1 ± 1.4 pg·µg protein$^{-1}$·10 min$^{-1}$ in mice fed 30 and 500 ppm vitamin E, respectively). Vitamin E, however, significantly decreased conversion of exogenous arachidonic acid to TXA$_2$ in LPS-stimulated macrophages of old mice (19.5 ± 4.7 and 7.8 ± 1.4 pg·µg protein$^{-1}$·10 min$^{-1}$ in mice fed 30 and 500 ppm vitamin E, respectively). Similar to the results for PGE$_2$ production, macrophages from old mice fed 30 ppm vitamin E had significantly higher TXA$_2$ production than young mice fed 30 ppm vitamin E. The magnitude of the inhibition of COX activity by vitamin E in old mice is similar whether measured as a conversion to PGE$_2$ or to TXA$_2$, i.e., ~60%. This further supports our conclusion that the effect of vitamin E is mediated through the reduction of COX activity rather than upstream or downstream enzymes.

In many cases, the altered COX activity reflects changes in the rate of enzyme synthesis, the rate of mRNA transcription, or both. Thus, to further clarify how vitamin E modifies COX activity in old mice, we examined COX protein and mRNA expression. In agreement with previous reports (9, 16, 24, 32) and as shown in Fig. 3, the expression of COX-2 mRNA reached a maximum after 2–4 h of stimulation with LPS. In contrast, COX-1 mRNA was barely detectable, and LPS stimulation did not potentiate its expression (data not shown). Accordingly, Western blot showed a low but detectable level of COX-1 protein, whereas COX-2 protein was detected in a substantial amount after 6 h of LPS stimulation. Our results are in concordance with previous studies showing that COX-1 is not activated...
by the different stimuli in such monocytoic cells as mouse peritoneal macrophages (35), rat alveolar macrophages (23), mouse RAW 264.7 macrophage cell line (35), and human monocytes and alveolar macrophages (18). In contrast, COX-2 mRNA and protein were barely detected in unstimulated cells but were induced by LPS (10, 23, 44). There was no significant effect of vitamin E on protein (as measured by Western blot analysis and immunoprecipitation) or mRNA level for either COX-1 or COX-2. Thus the vitamin E-induced decrease in COX activity of macrophages from old mice is not due to its regulation of COX transcription or translation; rather, it appears that vitamin E exerts its effect posttranslationally.

COX activity requires the presence of oxidant hydroperoxides (17, 21, 41). Hemler and Lands (17) showed that the lag phase in attaining maximal COX activity was shortened or eliminated by endogenous or exogenous hydroperoxide, whereas antioxidant phenol and α-naphthol, as well as glutathione peroxidase, inhibited COX catalysis and caused an extended lag period. These studies indicated that sufficient hydroperoxide is required to fully activate COX. It is interesting to note that Kulmacz and Wang (21) showed that COX initiation in COX-2 requires a lower level of hydroperoxide than that required by COX-1, allowing prostaglandin synthesis by COX-2 to proceed at hydroperoxide levels that will not support COX catalysis by COX-1. Vitamin E is an effective biological antioxidant and chain-breaking free radical scavenger, and therefore vitamin E might attenuate COX activity by scavenging the oxidant hydroperoxide necessary for COX activation. The fact that vitamin E inhibits COX activity in old but not young mice supports this notion because many studies have indicated increased formation of lipid peroxides in different tissues of the aged animals. The free radical NO has drawn increasing attention for its role in a variety of cell functions. Several studies have indicated its involvement in regulation of COX activity and eicosanoid metabolism. Some investigators have suggested that NO stimulates COX activity via direct stimulation of the enzyme (40). We recently (3) showed that LPS-stimulated peritoneal macrophages from old mice produced more NO than those from young mice, and dietary supplementation with vitamin E reduced NO production in macrophages from old mice. Thus inhibition of COX activity by vitamin E might be mediated through reduction of NO production. Further studies are needed to determine the role of NO and other free radical species in vitamin E-induced suppression of COX activity in aged mice.

Sakamoto et al. (39) reported that phorbol 12-myristate 13-acetate or A-23187-stimulated PGE2 production and arachidonic acid release from rat macrophages were inhibited following 6 days of intraperitoneal injection of vitamin E. They suggested and later confirmed (37) that the reduced PGE2 production resulted from inhibition of phospholipase A2 activity and the subsequent decrease in arachidonic acid release by vitamin E. This may represent one of the mechanisms underlying the modulation of prostaglandin synthesis by vitamin E and might have contributed to the vitamin E-decreased accumulation of PGE2 production in old mice reported here. This is not likely, however, since as mentioned above the effect of vitamin E on PGE2 production is observed when substrate (arachidonic acid) is not limited (see PGE2 production and COX enzyme activity). Furthermore, the decrease in PGE2 accumulation (60%) corresponds to the 60% decrease in COX activity. In the above-mentioned studies, Sakamotos group did not examine COX activity or expression of COX protein and mRNA. In contrast to their previous report (37), in a recent paper Sakamoto et al. (38) reported no effect of vitamin E on LPS-stimulated PGE2 production, COX activity, COX-2 protein, or mRNA levels of rat macrophages. The reason for this discrepancy is not clear. The age of the animals in the two studies by Sakamoto et al. (37, 38) was not defined. Because we observe the effect of vitamin E in old mice and not in young mice, one may speculate that there might have been age differences between the two studies of Sakamoto et al. (37, 38).

The age-associated increase in COX activity and PGE2 production and its reduction by vitamin E has significant biological and clinical implications for the aged. We, along with other groups, have shown that increased PGE2 production contributes to decreased T cell-mediated function with aging (2, 4, 11, 15, 26, 28, 36). Furthermore, we demonstrated that inhibition of PGE2 production by vitamin E can result in significant improvement in T cell proliferation, IL-2 production, delayed-type hypersensitivity skin response, and antibody production in aged mice and humans (4, 26–28). In addition to changes in T cell-mediated function with aging, there is dysregulation of other components of the immune system, resulting in increased inflammation and greater incidence of autoimmune diseases. Elderly humans and aged mice have higher IL-6 production than their young counterparts (7). Hinson et al. (20) showed that PGE3 induces IL-6 production in vivo. It is interesting to note that we previously showed that vitamin E supplementation significantly decreases stimulated IL-6 production by human peripheral blood mononuclear cells (6) and spontaneous IL-6 production by peritoneal macrophages of old mice (46). Furthermore, increased PGE2 production and COX activity have been implicated in the pathogenesis of several age-associated diseases such as arthritis, atherosclerosis, cancer, and autoimmune diseases. A beneficial effect of vitamin E in reducing the risk of cancer and heart diseases has been reported (25).

In summary, our results show that the age-associated increase in PGE2 production is reversed by vitamin E. Vitamin E exerts its effect posttranslationally through inhibition of COX activity. These findings can have significant implications for the development of preventive and therapeutic strategies to reduce age-associated pathogenesis, particularly that of immune and inflammatory diseases.

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