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Effects of Vitamin E and Prostaglandin E_2 on Expression of CREB1 and CREB2 Proteins by Human T Lymphocytes

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Summary

Both prostaglandins (PGs) and vitamin E are known to deeply affect immune responses. It is shown here that they both influence T cell-mediated immunity through reciprocal interference on the expression of cyclic-AMP responsive element binding (CREB) family proteins. CREB1 protein of human T lymphocytes was significantly modulated by a brief treatment of 5 to 10 min with PGE_2. On the contrary, vitamin E appeared to be ineffective on the CREB1 behavior, while it abolished the PGE_2-induced modulation of this protein. The CREB2 protein expression was also affected by PGE_2 treatment, but a longer period of incubation (>20 min) was needed to observe these changes. Vitamin E showed a strong enhancing effect on CREB2 that was partially reversed by the subsequent treatment with PGE_2. Our results support the idea that there is reciprocal interference between PGE_2 and vitamin E on PGE_2-induced signals in T lymphocytes. These data are in agreement with the reports concerning different cell systems and experimental conditions.

Key words

T lymphocytes • vitamin E • PGE_2 • CREB proteins

Introduction

Vitamin E exhibits dramatic effects on immune responses in addition to its numerous biological activities. In fact, it is able to modify the decline of immunological vigor in aging animals and humans activities (Gillis *et al.* 1981, Bash 1983, Meydani *et al.* 1986, 1990, Miller 1994). Age-associated depression of the immune system is related to a decline in T cell-mediated immunity (Vie and Miller 1986, Hallgren *et al.* 1988, Nagel *et al.* 1988, Ernst *et al.* 1989, Miller 1989, Kubo and Cinader 1990, Hobbs *et al.* 1991, Miller 1991, Karivi *et al.* 1992) due to both the intrinsic changes in the T cells themselves or the suppressive factors, such as PGE_2 (Goodwin *et al.* 1974, 1979, Gordon *et al.* 1976, Goodwin and Messner 1980, Webb *et al.* 1979, Bartocci *et al.* 1982, Goodwin and Ceuppens 1982, Minakuchi *et al.* 1990, Phupps *et al.* 1991, Anastassiou *et al.* 1992, Gold *et al.* 1994). The antioxidant property of vitamin E may be related to its immunostimulatory effect, exerted either by inhibiting PGE_2 synthesis or, under some experimental conditions, by a direct action on immunocompetent cells able to reverse the PGE_2 effects (Mariguchi *et al.* 1993, Beharka *et al.* 1997, Martin *et al.* 1997). The CREB proteins, a family of transcription factors phosphorylated at serine residues by protein kinase A (PKA), are enzymes involved in PGE_2-induced signal. This phosphorylation induces their ability to stimulate the transcription of the genes containing CRE elements (Yamamoto *et al.* 1988, Gonzalez and Montminy 1989). It has recently been

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reported that vitamin E has important effects on protein kinase activities (Tran *et al.* 1996, Studer *et al.* 1997, Maehira *et al.* 1998). We demonstrated that PGE_2 was able to induce the binding activity to CRE elements in human T lymphocytes (Micali *et al.* 1996). In this paper we studied the possible implications of vitamin E and PGE_2 on the behavior of CREB proteins in human peripheral T lymphocytes.

Methods

T cell preparation.

Human venous blood from eight healthy adult volunteers, collected with disposable syringes and anticoagulated with 10 units of heparin per ml, was used to isolate T lymphocytes. One hundred ml of whole blood were diluted with 150 ml of RPMI 1640 (Sigma, St. Louis, MO, USA) and then layered in centrifuge tubes onto 120 ml of Histopaque-1077 (Sigma) gradient. After centrifugation, the opaque interface containing monocytes, T and B cells was collected, washed twice in RPMI 1640, and after complete supernatant removal, the pellet was resuspended in PBS supplemented with 0.5 % of bovine serum albumin and 2 mM EDTA. T lymphocytes were magnetically separated by means of colloidal paramagnetic micro-beads conjugated to monoclonal mouse antibody anti-CD19 surface antigen expressed on macrophages, monocytes and B lineage cells using positive selection columns (Miltenyi Biotec, Bergisch Gladbach, Germany). The recovered T lymphocytes were tested by cytofluorimetric analysis using an anti-CD3 monoclonal antibody (Sigma-Aldrich, Milan, Italy). All samples contained >98 % T cells.

T cell treatment.

Purified T cells resuspended in RPMI 1640 (10^6/ml) were incubated at 25 °C with PGE_2 (Sigma) dissolved in ethanol to a final concentration of 10^{-4} M for 5,10 and 20 min, or with DL-alpha-tocopherol (vitamin E) (Fluka Chemie AG, Buchs, Switzerland) dissolved in ethanol/propylene glycol (1:9) to a final concentration of 10 mg/ml for 4 h, or preincubated with vitamin E and then treated with PGE_2 as above and washed.

Protein extracts

Protein extracts were prepared according to Li *et al.* (1991). Cells were lysed on ice in 100 µl of a buffer containing 10 mM Hepes, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.5 mM PMSF, pH 7.9, and passed approximately 10 times through a 28-gauge needle. The supernatants were transferred into new tubes after being spinned at 13 000 rpm for 10 min at 4 °C and the pellet was reextracted with 50 µl of a buffer (20 mM Hepes, 25% glycerol, 402 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 1.5 mM $MgCl_2$). The second supernatant was mixed with the preceding one. The protein concentrations of extracts were determined by Bio-Rad protein assay according to the method of Bradford (1976) and ranged from 0.5 to 1 mg/ml.

Immunoblot analysis

Protein extracts were analyzed by Western blot using an anti-CREB1 or an anti-CREB2 rabbit polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and visualized by the enhanced chemiluminescence system (Hybond ECL, Amersham International plc, Little Chalfont, England) using an anti-rabbit Ig horseradish peroxidase linked whole antibody (Amersham) as a secondary antibody and RPN 2209 ECL as luminiscence detector.

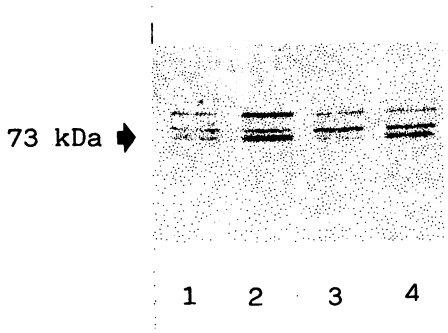

Fig. 1. *Effect of PGE_2 on CREB1 expression in human T lymphocytes. Lane 1: control (untreated cells). Lane 2: cells treated with PGE_2 (10^{-4} M) for 5 min. Lane 3: cells treated with PGE_2 (10^{-4} M) for 10 min. Lane 4: cells treated with PGE_2 (10^{-4} M) for 20 min. Blots shown are representative for four separate experiments.*

Results

Figure 1 shows the kinetics of CREB1 expression in human T lymphocytes after PGE_2 treatment. The lower band of the 73 kDa CREB1 isoform was more evident than the upper band 5 min after treatment, with respect to the control in which the two bands were similar. Ten minutes after incubation, a shift of the lower band could be observed, since the upper

band was more distinct than the former. Equilibrium between the two bands was reached 20 min after the incubation with PGE_2.

The effects of vitamin E treatment of T cells (4 h) are demonstrated in Figure 2. Vitamin E did not modify the expression of CREB1, since the two bands exhibited the same intensity and position relative to the control. When the incubation with vitamin E was followed by PGE_2 treatment, the lower band was less evident than the upper one and the effect seen 5 min after PGE_2 treatment was completely abolished.

The effect of PGE_2 on CREB2 protein expression is shown in Figure 3. It is evident that the 33-kDa band was not detectable in untreated control T lymphocytes and in T cells treated for 5 min with PGE_2.

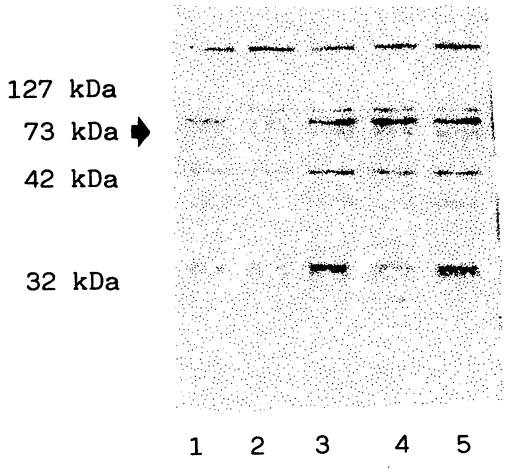

Fig. 2. *Effect of vitamin E and vitamin E plus PGE_2 treatment on CREB1 expression in human T lymphocytes. Lane 1: control (untreated cells). Lane 2: cells treated with vitamin E (10 mg/ml) for 4 hours. Lane 3: cells treated with vitamin E (10 mg/ml) for 4 h and PGE_2 (10^{-4} M) for 5 min. Lane 4: cells treated with vitamin E (10 mg/ml) for 4 h and PGE_2 (10^{-4} M) for 10 min. Lane 5: cells treated with vitamin E (10 mg/ml) for 4 hours and PGE_2 (10^{-4} M) for 20 min. Blots shown are representative for four separate experiments.*

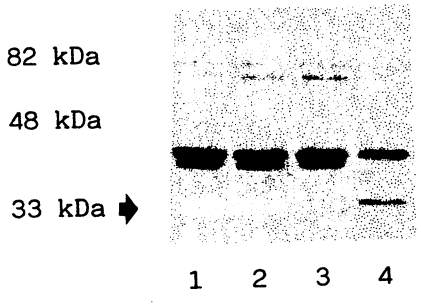

Fig. 3. *Effect of PGE_2 on CREB2 expression in human T lymphocytes. Lane 1: control (untreated cells). Lane 2: cells treated with PGE_2 (10^{-4} M) for 5 min. Lane 3: cells treated with PGE_2 (10^{-4} M) for 10 min. Lane 4: cells treated with PGE_2 (10^{-4} M) for 20 min. Blots shown are representative for four separate experiments.*

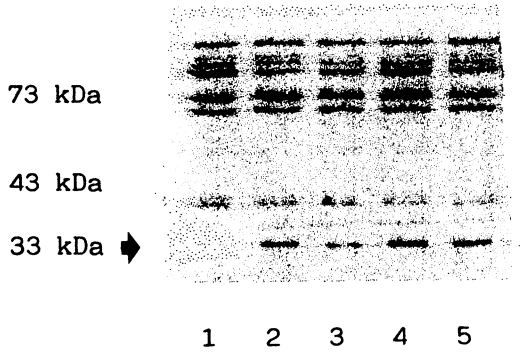

Fig. 4. *Effect of vitamin E and vitamin E plus PGE_2 treatment on CREB2 expression in human T lymphocytes. Lane 1: control (untreated cells). Lane 2: cells treated with vitamin E (10 mg/ml) for 4 hours. Lane 3: cells treated with vitamin E (10 mg/ml) for 4 h and PGE_2 10^{-4} M for 5 min. Lane 4: cells treated with vitamin E (10 mg/ml) for 4 h and PGE_2 (10^{-4} M) for 10 min. Lane 5: cells treated with vitamin E (10 mg/ml) for 4 h and PGE_2 10^{-4} M for 20 min. Blots shown are representative for four separate experiments.*

On the contrary, a band corresponding to CREB2 protein was evident 10 min after PGE_2 incubation and its intensity increased markedly in 20 min.

Figure 4 shows the effects of treatment with vitamin E alone which are followed by PGE_2 on CREB2 expression in T lymphocytes. Vitamin E induced a strong expression of the CREB2 protein, while the following treatment with PGE_2 for 5 min partially abolished the vitamin E-induced expression of this protein. The counteracting effect of PGE_2 on CREB2 expression induced by vitamin E could not be observed if the PGE_2 treatment was prolonged by 10-30 min.

Discussion

Our data clearly demonstrate that PGE_2 alone and vitamin E plus PGE_2 treatment of human T lymphocytes strongly affect the expression of the two

members of CREB proteins studied. In particular, PGE_2 was able to modulate the behavior of the 73 kDa CREB1 isoforms within a very short period of time (5 to 10 min of incubation). Of the two bands detected in control T cells, the lower band became more evident after 5 min incubation, while the upper band was most marked after 10 min, indicating a shift of the bands, presumably as a result of phosphorylation. On the contrary, vitamin E alone had no effect on CREB1 expression. However, if the treatment was followed with PGE_2, it abolished the PGE_2-induced modulation of this protein. On the other hand, the CREB2 expression was greatly affected by the incubation of T cells with PGE_2, since the 33 kDa band relative to this protein was absent in control untreated cells and became detectable only 10 min after the treatment. This behavior may indicate a mobilization and/or an activation of the protein because of the brief period of PGE_2 incubation required for CREB2 induction. Treatment with vitamin E alone strongly affects CREB2 expression, in contrast with its negligible effect on CREB1. The second incubation with PGE_2 partially reverses the vitamin E enhancing action on CREB2 expression. The modulating effect of PGE_2 was particularly evident after 5 min of T lymphocyte incubation. The reciprocal reverse action of vitamin E and PGE_2 presented here is in agreement with literary data, which report a similar effect on several cell functions as well as responsivity to a variety of stimuli (Beharka *et al.* 1997, Yano *et al.* 1997). Meydani *et al.* (1986, 1990) hypothesized that the increased production of PGE_2 would contribute to decreased T cells function with aging and that dietary antioxidants, such as vitamin E, enhance T cell-mediated immunity by decreasing macrophage PGE_2 production. However, the inter-relations between vitamin E and PGE_2 appear to be more complex, since it has been demonstrated that vitamin E, under some experimental conditions, may have a direct effect on T cells independent of its effect on macrophages (Beharka *et al.* 1997).

It has been demonstrated that exogenous PGE_2 overcomes the effect of vitamin E on ornithine decarboxylase activity in urethane-induced lung tumorigenesis in mice (Yano *et al.* 1997a). Similar events appear to occur in our experimental conditions. In fact, the addition of PGE_2 to vitamin E-treated T lymphocytes reversed the effect of vitamin on CREB2 expression and it was ineffective on the CREB1 modulation induced by PGE_2 alone. Moreover, Beharka *et al.* (1997) reported that, in addition to its inhibition of PGE_2 production, vitamin E enhanced the T-cell-mediated functions in old mice through other mechanisms involving impairment of the PGE_2-induced signal.

PGE_2 effects on T lymphocytes appeared to be mediated by receptor binding followed by accumulation of cAMP, which activated PKA (Ramer *et al.* 1991). PGE_2 and other cAMP-elevating agents have been shown to decrease phosphatidylinositol (PI) turnover and to inhibit phytohemoagglutinin (PHA) and anti-CD3-stimulated increase in Ca^{2+} (Bismuth *et al.* 1988, Papadogiannakis *et al.* 1989). On the contrary, cAMP levels were not influenced by supplementation with vitamin E in BL6 cells (Ottino and Duncan 1997).

Vitamin E seems to have contrasting effects on PKC, presumably because of differences in the cell systems involved. In fact, alpha-tocopherol blocked the increase of protein kinase C (PKC) induced by glucose, tromboxane analog U46619 and angiotensin II (Studer *et al.* 1997) in mesangial cells, while it enhanced the activity of acid cholesteryl ester hydrolases (ACEH) through PKC activation in the rat aorta (Maehira *et al.* 1998). In heart myoblastic cells, vitamin E treatment by itself did not affect the PKC activity (Tran *et al.* 1996).

Taken together, these data support the hypothesis that the correlation between vitamin E and PGE_2 is numerous and complex and goes beyond the antioxidant action of vitamin E and its effect on PGE_2 production. Even though this latter effect cannot be excluded in our experiments, since the vitamin E treatment lasted for 4 h, the addition of exogenous PGE_2 on vitamin-treated T cells can account for a reciprocal interference of these two mediators on CREB protein family behavior.

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