

1,25-Dihydroxyvitamin D₃ enhances prostaglandin E₂ production by monocytes

A mechanism which partially accounts for the antiproliferative effect of 1,25(OH)₂D₃ on lymphocytes

R. Koren, A. Ravid⁺, C. Rotem, E. Shohami*, U.A. Liberman and A. Novogrodsky⁺

*The Unit of Metabolic Diseases, ⁺ Rogoff-Wellcome Medical Research Institute, Beilinson Medical Center, Petah-Tikva and *Department of Pharmacology, The Hebrew University, Hadassah Medical School, Jerusalem, Israel*

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Partial removal of monocytes from human peripheral blood mononuclear cells, or the addition of indomethacin, reduced the antiproliferative effect of 1,25(OH)₂D₃ on mitogen-stimulated mononuclear cells. Addition of 1,25(OH)₂D₃ (1 nM) to mitogen-stimulated mononuclear cells caused a 2–4-fold increase in prostaglandin E₂ production during the second day of culture. The inhibitory effect of 1,25(OH)₂D₃ on lymphocyte proliferation is greatly augmented up to 7-fold in the presence of prostaglandin E₂. We conclude that monocytes are involved in the inhibitory effect of 1,25(OH)₂D₃ on the mitogenic stimulation of human lymphocytes and that their action is probably mediated by prostaglandins.

1,25-Dihydroxyvitamin D₃ Prostaglandin E Monocyte Lymphocyte activation

1. INTRODUCTION

1,25(OH)₂D₃ induces the differentiation and maturation of malignant and normal cells of the monocytic lineage at various stages of their development [1]. 1,25(OH)₂D₃ enhances macrophage functions like oxygen radical formation, phagocytosis and lymphokine-induced monokine production [1,2]. The hormone inhibits mitogen and antigen-induced T cell proliferation and antibody secretion by B cells [3–6]. The inhibitory effect of 1,25(OH)₂D₃ on T cell activation is associated with inhibition of both the production and the response to interleukin 2 [3,4] and is dependent upon the

presence of high-affinity receptors for 1,25(OH)₂D₃ [7]. Receptors for 1,25(OH)₂D₃ are present in monocytes and in mitogen-activated peripheral blood human lymphocytes [8]. The proliferative response of T cells to mitogens in a PBM cell preparation involves the interaction of accessory cells, mainly monocytes, with the lymphocytes. However, monocytes may produce factors like prostaglandins, leukotrienes and oxygen radicals which inhibit lymphocyte proliferation [9,10]. The inhibition of mitogen-induced PBM cell stimulation by 1,25(OH)₂D₃ may be mediated by effects on the regulatory monocytes and/or the effector T lymphocytes.

Abbreviations: 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; PHA, phytohemagglutinin; ConA, concanavalin A; PGE₂, prostaglandin E₂; PBM, peripheral blood mononuclear

2. MATERIALS AND METHODS

2.1. *Materials*

Purified PHA was obtained from Wellcome Re-

search Laboratories, Bekenham, England. ConA, $2 \times$ crystallized was from Bio Yeda, Rehovot, Israel. $1,25(\text{OH})_2\text{D}_3$ was obtained from Hoffmann-LaRoche, Nutley, NJ (a gift from Dr M. Uskokovic). Indomethacin and PGE_2 were from Sigma Chemicals. [*methyl*- ^3H]Thymidine (2 Ci/mmol) was from Nuclear Research Center Negev, Beer-Sheva, Israel and [^3H] PGE_2 from New England Nuclear, Boston, MA, USA.

2.2. Cell preparation and culture conditions

Buffy coats from the Beilinson Medical Center Blood Bank were used as a source for human PBM cells. PBM cells were separated by Ficoll-Hypaque density gradient centrifugation [7]. Partial depletion of adherent cells was achieved by incubating PBM cells ($5 \times 10^6/\text{ml}$) for 90 min at 37°C in RPMI-1640 medium containing 2% heat-inactivated fetal calf serum in plastic petri dishes. Monocyte content of PBM cells was thus reduced from ~ 20 to $\sim 6\%$ and the yield of the non-adherent cells was about 50%. Cell culture and mitogenic stimulation conditions were as described [7]. PHA ($1 \mu\text{g}/\text{ml}$), ConA ($10 \mu\text{g}/\text{ml}$), indomethacin, $1,25(\text{OH})_2\text{D}_3$ and PGE_2 were added at the initiation of the culture. [^3H]Thymidine ($1 \mu\text{Ci}/\text{well}$) was added at 68 h and the cells were harvested 4 h later. PGE_2 was determined in conditioned media obtained by culturing PBM cells ($2 \times 10^6/\text{ml}$) in growth medium for 48 h. ConA ($10 \mu\text{g}/\text{ml}$) or PHA ($1 \mu\text{g}/\text{ml}$) or $1,25(\text{OH})_2\text{D}_3$ (1 nM) were added at zero time. Indomethacin ($10 \mu\text{g}/\text{ml}$) was added at zero time or at 24 h. Cells were cultured in 24 well plates ($1 \text{ ml}/\text{well}$).

2.3. Determination of PGE_2

PGE_2 was determined in 0.1 ml aliquots of conditioned media by radioimmunoassay as described [11], using a specific rat antibody that has a cross-reactivity of $< 1\%$ with all major PGs (Bio Yeda, Rehovot, Israel).

3. RESULTS

PBM cells were stimulated with PHA in the presence or absence of $1,25(\text{OH})_2\text{D}_3$ (1 nM). The hormone significantly inhibited the mitogen-induced DNA synthesis (fig.1). Partial removal of adherent cells resulted in a decrease in the inhibitory effect of the hormone in nine out of

twelve donors. The extent of inhibition for the twelve donors was reduced from 42 ± 2.9 to $22 \pm 3.9\%$ (mean $\pm$ SE) (fig.1). Adherent cells may participate in the $1,25(\text{OH})_2\text{D}_3$ -dependent inhibition of lymphocyte proliferation by the production of PGE . This thesis was investigated by studying the effect of $1,25(\text{OH})_2\text{D}_3$ in the presence or absence of the cyclooxygenase inhibitor indomethacin. The inhibitor was used at a concentration ($5 \mu\text{g}/\text{ml}$) which was found, in preliminary experiments, to inhibit PGE_2 production by more than 90%. Indomethacin did not affect PHA-induced mitogenic stimulation of PBM cells, but

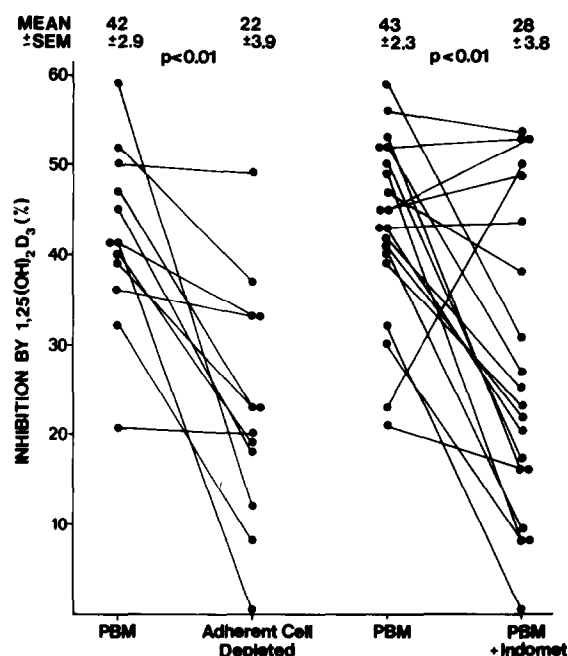


Fig. 1. The effect of adherent cell depletion and indomethacin on the response of PBM cells to $1,25(\text{OH})_2\text{D}_3$. Mononuclear cells were partially depleted of adherent cells and cultured for 72 h with PHA ($1 \mu\text{g}/\text{ml}$) in presence or absence of $1,25(\text{OH})_2\text{D}_3$ (1 nM) (left panel). In another series of experiments, PHA-stimulated PBM cells were cultured in the presence or absence of indomethacin ($5 \mu\text{g}/\text{ml}$) and/or $1,25(\text{OH})_2\text{D}_3$ (1 nM). All experiments were done in triplicate cultures. The effect of $1,25(\text{OH})_2\text{D}_3$ on thymidine incorporation in treated cultures was expressed as percent inhibition of control cultures. Each line represents a different experiment performed on cells from a different donor.

Statistical analysis was done by paired *t*-test.

significantly ($p < 0.01$) reduced the inhibitory effect of $1,25(\text{OH})_2\text{D}_3$ from 43 ± 2.3 to $28 \pm 3.8\%$ (fig.1). No such effect was observed in cultures depleted of adherent cells (not shown). A similar effect of indomethacin was observed when ConA served as a mitogen (inhibition of DNA synthesis was reduced from 53 ± 4 to $36 \pm 6\%$, mean $\pm$ SE). We conclude from these results that adherent cells are involved in the inhibitory action of $1,25(\text{OH})_2\text{D}_3$ on lymphocyte proliferation probably via indomethacin-sensitive pathway(s).

The next experiments were performed in order to find out whether $1,25(\text{OH})_2\text{D}_3$ affects the production of PGE_2 . PGE_2 concentrations in 48 h cultures of mitogen-stimulated PBM cells are significantly higher in the presence of $1,25(\text{OH})_2\text{D}_3$ (table 1). The addition of indomethacin ($10 \mu\text{g/ml}$) at the initiation of cultures abolished the production of PGE_2 . Assuming that the addition of indomethacin at 24 h blocks PGE_2 production in the second day of culture, PGE_2 accumulation during the first day can be determined directly and during the second day calculated by subtraction. No effect of $1,25(\text{OH})_2\text{D}_3$ on PGE_2 accumulated during the first day of culture could be observed (table 1). On the other hand, $1,25(\text{OH})_2\text{D}_3$ increased the ac-

cumulation of PGE_2 during the second day of culture from 1.3 to $5.3 \text{ ng}/10^6$ cells in the ConA and from 1.6 to $3.4 \text{ ng}/10^6$ cells in the PHA-stimulation systems. To find out whether $1,25(\text{OH})_2\text{D}_3$ -dependent inhibition of lymphocyte proliferation was affected by PGE_2 , PBM cells depleted of adherent cells were stimulated with optimal concentration of PHA in the presence of various concentrations of PGE_2 and $1,25(\text{OH})_2\text{D}_3$ (fig.2). Under these experimental conditions PGE_2 alone did not affect the rate of DNA synthesis. However, the presence of PGE_2 potentiated up to 7-fold the inhibitory effect of $1,25(\text{OH})_2\text{D}_3$.

4. DISCUSSION

The results of this work support the notion that monocytes, probably via the production of prostaglandins, are responsible at least partially for the inhibitory effect of $1,25(\text{OH})_2\text{D}_3$ on the mitogen-stimulated human PBM cells. This conclusion is based upon the following findings: partial depletion of monocytes, by plastic adherence, resulted in a diminished inhibitory effect of $1,25(\text{OH})_2\text{D}_3$ on lymphocyte proliferation. A similar effect was produced by blocking prostaglandin synthesis in PBM cell cultures, but not in cultures depleted of monocytes.

Table 1

Additions		PGE_2 (ng/ 10^6 cells) in conditioned media of PBM cells cultured with	
0 h	24 h	None	$1,25(\text{OH})_2\text{D}_3$
ConA	—	2.21 ± 0.39	6.41 ± 1.65 ($p < 0.01$) ^b
ConA + indomet	—	0.02	ND
ConA	indomet	0.89 ± 0.10	1.12 ± 0.39 (NS)
PHA	—	2.52 ± 0.63	4.64 ± 0.48 ($p < 0.01$)
PHA + indomet	—	ND ^a	0.07
PHA	indomet	0.92 ± 0.09	1.28 ± 0.70 (NS)

^aND, non-detectable

^bSignificance of the effect of $1,25(\text{OH})_2\text{D}_3$ as judged by the unpaired *t*-test. NS, non-significant
PBM cells were cultured for 48 h in the presence of mitogen. $1,25(\text{OH})_2\text{D}_3$ (1 nM) was added at time zero and indomethacin ($10 \mu\text{g/ml}$) at time zero or at 24 h. All cultures were performed in quadruplicates and the results presented as mean $\pm$ SD

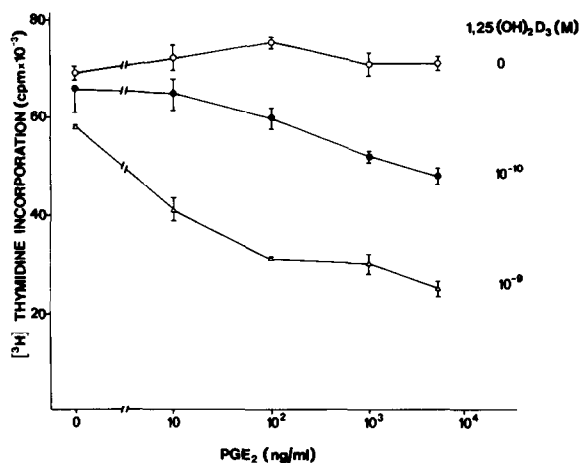


Fig.2. The effect of $1,25(\text{OH})_2\text{D}_3$ and PGE_2 on lymphocyte proliferation. PBM cells were partially depleted of adherent cells and cultured in the presence of PHA and various concentrations of PGE_2 and/or $1,25(\text{OH})_2\text{D}_3$. The results are presented as the mean $\pm$ SD of triplicate cultures.

Prostaglandins may be involved in the mediation of $1,25(\text{OH})_2\text{D}_3$ action by two mechanisms: (i) $1,25(\text{OH})_2\text{D}_3$ may enhance the production of monocyte-derived prostaglandins; (ii) $1,25(\text{OH})_2\text{D}_3$ and prostaglandins may act synergistically to inhibit lymphocyte stimulation. Our findings that $1,25(\text{OH})_2\text{D}_3$ increases the production of PGE_2 and that its effect is potentiated by PGE_2 demonstrate that both mechanisms may operate. Furthermore, the concentrations of PGE_2 produced in the presence of $1,25(\text{OH})_2\text{D}_3$ are of the same order of magnitude as those which potentiate the effect of $1,25(\text{OH})_2\text{D}_3$. PGE_2 is known to activate adenylate cyclase. Although the mechanism of the cooperation between PGE_2 and $1,25(\text{OH})_2\text{D}_3$ is not known, it most probably does not involve a $1,25(\text{OH})_2\text{D}_3$ -mediated increase in cyclic AMP production, since it has been shown that $1,25(\text{OH})_2\text{D}_3$ attenuates rather than increases cyclic AMP accumulation in activated human T lymphocytes [12].

The interactions between $1,25(\text{OH})_2\text{D}_3$ and PGE_2 demonstrated in this study may suggest that the hormone has a modulatory role in other processes which involve prostaglandins, like inflammation. The finding that $1,25(\text{OH})_2\text{D}_3$ enhances the production of PGE_2 by monocytes may have a direct bearing on the well documented bone resorbing activity of the hormone, since there is evidence that monocytes stimulate osteoclastic bone resorption, an effect mediated by prostaglandins [13].

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REFERENCES

- [1] Gray, T.K. and Cohen, M.S. (1985) *Surv. Immunol. Res.* 4, 200-212.
- [2] Amento, E.P., Bhalla, A.K., Kurnick, J.T., Kradin, R.Z., Clements, T.L., Holick, S.A., Holick, M.F. and Krane, S.M. (1984) *J. Clin. Invest.* 73, 731-739.
- [3] Tsoukas, C.D., Provvedini, D.M. and Manolagas, S.C. (1984) *Science* 224, 1438-1440.
- [4] Ravid, A., Koren, R., Novogrodsky, A. and Liberman, U.A. (1984) *Biochem. Biophys. Res. Commun.* 123, 163-169.
- [5] Bhalla, A.K., Amento, E.P., Serog, B. and Glimcher, L.H. (1984) *J. Immunol.* 133, 1748-1754.
- [6] Lemire, J.M., Adams, J.S., Sakai, R. and Jordan, S.C. (1984) *J. Clin. Invest.* 74, 657-661.
- [7] Koren, R., Ravid, A., Liberman, U.A., Hochberg, Z., Weisman, Y. and Novogrodsky, A. (1985) *J. Clin. Invest.* 76, 2012-2015.
- [8] Provvedini, D.M., Tsoukas, C.D., Deftos, L.J. and Manolagas, S.C. (1983) *Science* 221, 1181-1183.
- [9] Humes, J.L., Bonney, R.J., Pelus, L., Dahlgorn, M.E., Sadowski, S.J., Kuehl, F.A. and Davies, P. (1977) *Nature* 269, 149-151.
- [10] Goodwin, J.S., Bankhurst, A.D. and Messner, R.P. (1977) *J. Exp. Med.* 146, 1719-1734.
- [11] Shohami, E. and Gross, J. (1985) *J. Neurochem.* 45, 132-136.
- [12] Catherwood, B.D. and Rubin, J.E. (1985) *Clin. Res.* 33, 100A.
- [13] Dominguez, J.H. and Mundy, G.R. (1980) *Calcif. Tissue Int.* 31, 29-34.