1,25-Dihydroxyvitamin D_3 enhances prostaglandin E_2 production by monocytes

A mechanism which partially accounts for the antiproliferative effect of $1,25(OH)_2D_3$ 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*

Received 29 May 1986

Partial removal of monocytes from human peripheral blood mononuclear cells, or the addition of indomethacin, reduced the antiproliferative effect of $1,25(OH)_2D_3$ on mitogen-stimulated mononuclear cells. Addition of $1,25(OH)_2D_3$ (1 nM) to mitogen-stimulated mononuclear cells caused a 2–4-fold increase in prostaglandin E_2 production during the second day of culture. The inhibitory effect of $1,25(OH)_2D_3$ on lymphocyte proliferation is greatly augmented up to 7-fold in the presence of prostaglandin E_2. We conclude that monocytes are involved in the inhibitory effect of $1,25(OH)_2D_3$ on the mitogenic stimulation of human lymphocytes and that their action is probably mediated by prostaglandins.

1,25-Dihydroxyvitamin D_3 Prostaglandin E Monocyte Lymphocyte activation

1. INTRODUCTION

$1,25(OH)_2D_3$ induces the differentiation and maturation of malignant and normal cells of the monocytic lineage at various stages of their development [1]. $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ [7]. Receptors for $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ may be mediated by effects on the regulatory monocytes and/or the effector T lymphocytes.

Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; PHA, phytohemagglutinin; ConA, concanavalin A; PGE_2, prostaglandin E_2; PBM, peripheral blood mononuclear

2. MATERIALS AND METHODS

2.1. *Materials*

Purified PHA was obtained from Wellcome Re-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

search Laboratories, Bekenham, England. ConA, 2× crystallized was from Bio Yeda, Rehovot, Israel. $1,25(OH)_2D_3$ was obtained from Hoffmann-LaRoche, Nutley, NJ (a gift from Dr M. Uskokovic). Indomethacin and PGE_2 were from Sigma Chemicals. [*methyl*-^{3}H]Thymidine (2 Ci/mmol) was from Nuclear Research Center Negev, Beer-Sheva, Israel and [^{3}H]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 (5×10^6/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 ~6% and the yield of the non-adherent cells was about 50%. Cell culture and mitogenic stimulation conditions were as described [7]. PHA (1 μg/ml), ConA (10 μg/ml), indomethacin, $1,25(OH)_2D_3$ and PGE_2 were added at the initiation of the culture. [^{3}H]Thymidine (1 μCi/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 (2×10^6/ml) in growth medium for 48 h. ConA (10 μg/ml) or PHA (1 μg/ml) or $1,25(OH)_2D_3$ (1 nM) were added at zero time. Indomethacin (10 μg/ml) was added at zero time or at 24 h. Cells were cultured in 24 well plates (1 ml/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 $1,25(OH)_2D_3$ (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 ± 3.9% (mean ± SE) (fig.1). Adherent cells may participate in the $1,25(OH)_2D_3$-dependent inhibition of lymphocyte proliferation by the production of PGE. This thesis was investigated by studying the effect of $1,25(OH)_2D_3$ in the presence or absence of the cyclooxygenase inhibitor indomethacin. The inhibitor was used at a concentration (5 μg/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

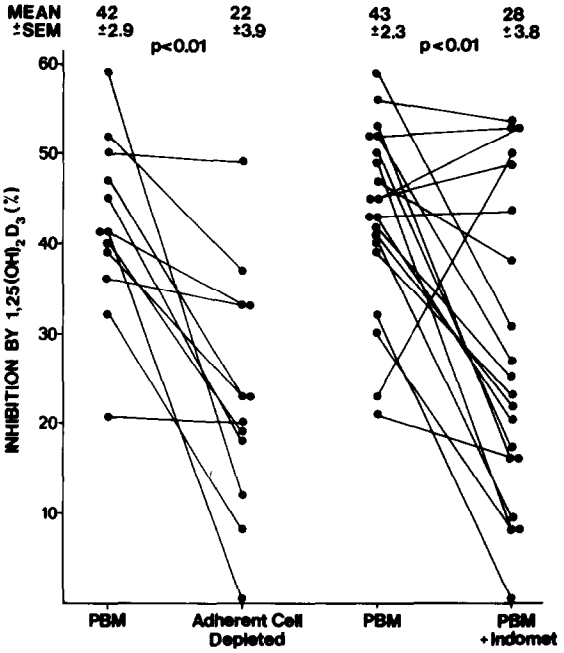

Fig. 1. The effect of adherent cell depletion and indomethacin on the response of PBM cells to $1,25(OH)_2D_3$. Mononuclear cells were partially depleted of adherent cells and cultured for 72 h with PHA (1 μg/ml) in presence or absence of $1,25(OH)_2D_3$ (1 nM) (left panel). In another series of experiments, PHA-stimulated PBM cells were cultured in the presence or absence of indomethacin (5 μg/ml) and/or $1,25(OH)_2D_3$ (1 nM). All experiments were done in triplicate cultures. The effect of $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ 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 ± SE). We conclude from these results that adherent cells are involved in the inhibitory action of $1,25(OH)_2D_3$ on lymphocyte proliferation probably via indomethacin-sensitive pathway(s).

The next experiments were performed in order to find out whether $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ (table 1). The addition of indomethacin (10 μg/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 $1,25(OH)_2D_3$ on PGE_2 accumulated during the first day of culture could be observed (table 1). On the other hand, $1,25(OH)_2D_3$ increased the accumulation of PGE_2 during the second day of culture from 1.3 to 5.3 ng/10^6 cells in the ConA and from 1.6 to 3.4 ng/10^6 cells in the PHA-stimulation systems. To find out whether $1,25(OH)_2D_3$-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 $1,25(OH)_2D_3$ (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 $1,25(OH)_2D_3$.

Table 1

Additions		PGE_2 (ng/10^6 cells) in conditioned media of PBM cells cultured with	
0 h	24 h	None	$1,25(OH)_2D_3$
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)

[a]ND, non-detectable
[b]Significance of the effect of $1,25(OH)_2D_3$ as judged by the unpaired *t*-test. NS, non-significant
PBM cells were cultured for 48 h in the presence of mitogen. $1,25(OH)_2D_3$ (1 nM) was added at time zero and indomethacin (10 μg/ml) at time zero or at 24 h. All cultures were performed in quadruplicates and the results presented as mean ± SD

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 $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ on lymphocyte proliferation. A similar effect was produced by blocking prostaglandin synthesis in PBM cell cultures, but not in cultures depleted of monocytes.

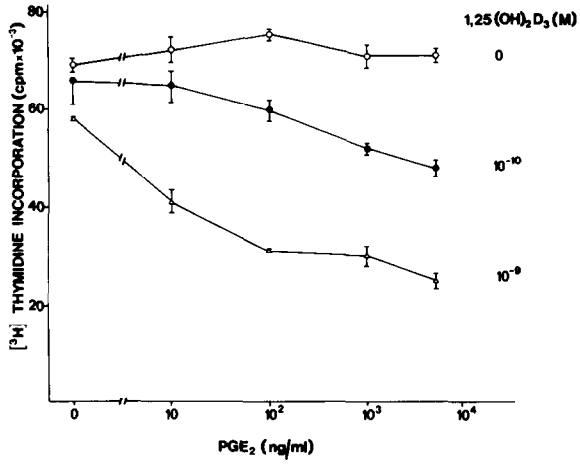

Fig.2. The effect of $1,25(OH)_2D_3$ and PGE_2 on lymphocyte proliferation. PBM cells were partially depleted of adherent cells and cultured in the presence of PHA and various concentrations of PGE_2 and/or $1,25(OH)_2D_3$. The results are presented as the mean ± SD of triplicate cultures.

Prostaglandins may be involved in the mediation of $1,25(OH)_2D_3$ action by two mechanisms: (i) $1,25(OH)_2D_3$ may enhance the production of monocyte-derived prostaglandins; (ii) $1,25(OH)_2D_3$ and prostaglandins may act synergistically to inhibit lymphocyte stimulation. Our findings that $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ are of the same order of magnitude as those which potentiate the effect of $1,25(OH)_2D_3$. PGE_2 is known to activate adenylate cyclase. Although the mechanism of the cooperation between PGE_2 and $1,25(OH)_2D_3$ is not known, it most probably does not involve a $1,25(OH)_2D_3$-mediated increase in cyclic AMP production, since it has been shown that $1,25(OH)_2D_3$ attenuates rather than increases cyclic AMP accumulation in activated human T lymphocytes [12].

The interactions between $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ enhances the production of PGE_2 by monocytes may have a direct bearing on the well documented bone resorbing acitivity of the hormone, since there is evidence that monocytes stimulate osteoclastic-bone resorption, an effect mediated by prostaglandins [13].

ACKNOWLEDGEMENTS

We wish to thank Dr Z. Keinan for his helpful assistance, the Beilinson Medical Center Blood Bank personnel for the supply of blood samples and Ms H. Reichel for her help in the preparation of the manuscript. This work was supported by the Stacher Memorial Research Fund and by a grant from the Israeli Ministry of Health.

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