1α,25-Dihydroxyvitamin D_3 (calcitriol) stimulates proliferation of human circulating monocytes in vitro

Masataka Ohta, Tetsuro Okabe, Keiya Ozawa, Akio Urabe and Fumimaro Takaku

The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Tokyo 113, Japan

Received 28 February 1985

Previous studies demonstrated that human circulating monocytes can proliferate in vitro when incubated with lectin-induced factor(s) from lymphocytes [(1985) Biochem. Biophys. Res. Commun., in press]. This study shows that human monocytes were induced to proliferate when incubated with 1α,25-dihydroxyvitamin D_3 (calcitriol) at physiological concentrations. The optimal dose was about 10 nM. Proliferative activity was examined both by measuring the [^{3}H]thymidine incorporation and by counting cell nuclei. Among other derivatives of vitamin D_3, 1α,24*R*-dihydroxyvitamin D_3 and 1α,24*R*,25-trihydroxyvitamin D_3 stimulated mitotic activity of monocytes. Addition of both calcitriol and lectin-stimulated lymphocyte-conditioned medium to the monocyte culture had an additional effect on the mitotic activity of monocytes.

1α,25-Dihydroxyvitamin D_3 *Calcitriol* *Vitamin D_3* *Monocyte* *Macrophage* *Monocyte-growth factor*

1. INTRODUCTION

It is not known whether human circulating monocytes are capable of undergoing cell division [2,3]. Since some studies reported that human circulating monocytes are minimally labeled with [^{3}H]thymidine [4,5], it has been generally accepted that they do not divide under normal circumstances. Van Furth et al. [5] suggested that precursors of monocytes in the bone marrow, promonocytes, actively divide but that human circulating monocytes are nondividing under normal circumstances. While studying an assay system for human circulating monocyte-growth factor (MoGF) [1], we found that 1α,25-dihydroxyvitamin D_3 (calcitriol), the naturally occurring hormonal form of vitamin D_3, induces the proliferation of human circulating monocytes in vitro.

Abbreviations: 1,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 1$(OH)D_3$, 1α-hydroxyvitamin D_3; 25$(OH)D_3$, 25-hydroxyvitamin D_3; 1,24$(OH)_2D_3$, 1α,24*R*-dihydroxyvitamin D_3; 1,24,25$(OH)_3D_3$, 1α,24*R*,25-trihydroxyvitamin D_3; MoGF, monocyte-growth factor; PHA, phytohemagglutinin antigen; PHA-LCM, PHA-stimulated lymphocyte-conditioned medium

2. MATERIALS AND METHODS

2.1. *Preparation and incubation of monocytes*

Monocytes were isolated and incubated as in [1,6], except that monocytes were isolated from mononuclear cells after overnight incubation in macrophage-separating plates (MSP; Jimro, Japan). Mononuclear cells from healthy adult volunteers were placed in macrophage-separating plates (10^7 cells/plates) in RPMI 1640 medium containing 10% autologous serum and incubated overnight in a chamber flushed with 5% CO_2-95% air at 37°C. Non-adherent cells were removed by repeated pipetting and washing. The adherent cells were treated with 0.02% EDTA dissolved in phosphate-buffered saline (PBS) for 30 min at 4°C. The remaining cells were harvested by vigorous pipetting and were washed twice with PBS. More than 98% of the harvested cells were monocytes, judged by positive α-naphthylbutyrate esterase reaction [7] and phagocytosis of latex particles [8]. The harvested cells were incubated (4×10^4/well; 24-well plate, Falcon 3047) in RPMI 1640 medium containing 10% autologous serum.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

2.2. *Measurement of mitotic activity of human monocytes*

Mitotic activity was examined by measuring [^{3}H]thymidine incorporation into cultured monocytes as reported [1,6]. After monocytes were pulse-labeled with [^{3}H]thymidine (0.5 μCi/well; spec. act. 6.7 Ci/mmol) for the last 18 h of incubation, radioactivity in the harvested cells was measured. Monocytes were incubated for 9 days unless stated otherwise. The nuclei of monocytes were counted as described [9]. All determinations were performed in triplicate cultures, and all experiments were performed at least 3 times.

2.3. *Chemicals, hormones and other materials*

Calcitriol and other derivatives of vitamin D_3 were gifts from Dr Y. Nishii, Chugai Pharma. Co. Ltd, and were dissolved in ethanol in the absence of oxygen. The final concentration of vehicle ethanol in culture medium was 0.5%. Addition of 0.5% ethanol to the monocyte culture had no significant effect on the mitotic activity. Testosterone, prostaglandin E_1, dexamethasone and retinoic acid were purchased from Sigma, St. Louis, USA. Human parathyroid hormone (1–44) and human calcitonin were purchased from Peptide Institute, Osaka, Japan. PHA-stimulated human lymphocyte-conditioned medium (PHA-LCM) was prepared as reported [1].

3. RESULTS AND DISCUSSION

Calcitriol induced the proliferation of monocytes in culture (fig.1). The optimal effect appears to be at about 10 nM, as shown in fig.2. Monocytes incubated with 10 nM calcitriol began to show enhanced [^{3}H]thymidine incorporation after the fourth day of culture and maximal [^{3}H]thymidine uptake was observed on the ninth day (fig.1A). The number of cell nuclei increased up to 3-times that of the control culture on the tenth day (fig.1B). Mitotic figures of the large adherent cells were frequently observed during the second week of the culture. More than 99.0% of the adherent cells on the ninth day of culture were macrophages, as judged by positive α-naphthylbutyrate esterase reaction and phagocytosis of latex particles.

Proliferation of monocytes was also observed by the addition of a physiological concentration

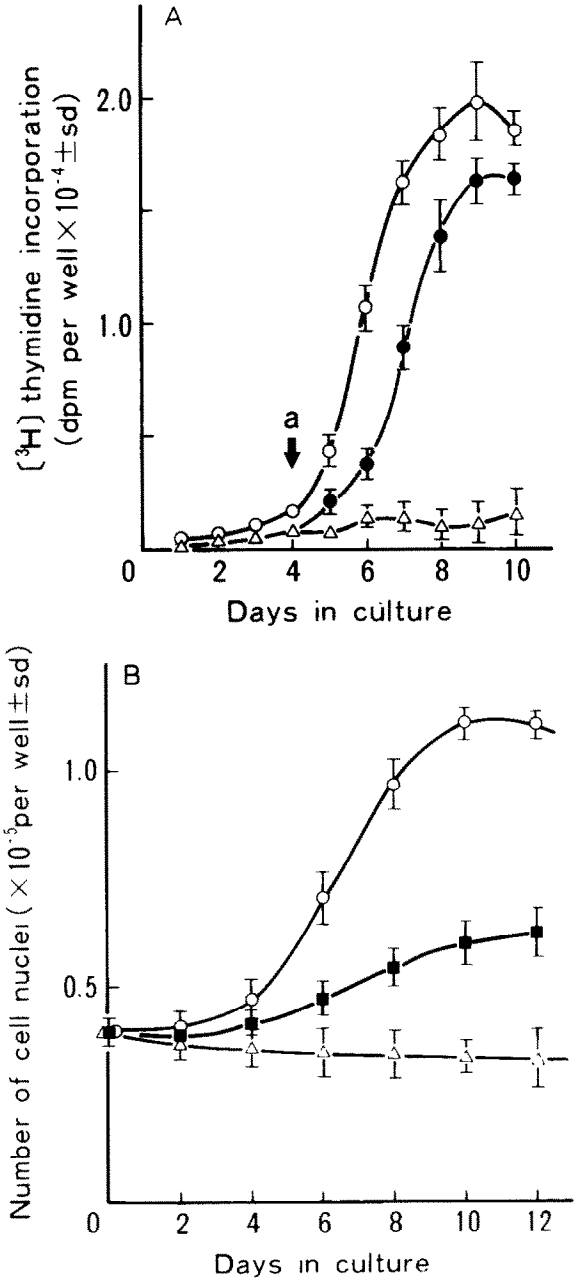

Fig.1. Time course of proliferation of monocytes in culture. Monocytes were incubated for up to 12 days: (A) [^{3}H]thymidine incorporation into adherent cells was counted every day and (B) cell nuclei were counted every other day in the presence of 0.1 nM (■), 10 nM (○) or vehicle (0.5% ethanol) (△). In another experiment, 10 nM calcitriol was added to the monocytes that were incubated for 4 days without calcitriol (**a**) and [^{3}H]thymidine incorporation (●) was measured every day. Data are means ± SD of triplicate cultures of one experiment.

(0.1 nM) of calcitriol (figs 1B and 2). However, there was no significant increase in the number of cell nuclei in the control cultures without calcitriol, although a small increase in the [^{3}H]thymidine uptake was observed. This increase may be attributable to endogenous calcitriol in the autologous serum used for the monocyte culture, since the promoted uptake of [^{3}H]thymidine was observed at as little as 0.01 nM, about one tenth of the serum calcitriol concentration.

Fig.2 demonstrates the dose effect of calcitriol and PHA-LCM. Calcitriol and PHA-LCM appeared to have optimal effects at 10 nM and 20%, respectively. PHA-LCM promoted the mitotic activity of monocytes even in the presence of an optimal dose (10 nM) of calcitriol.

Among the derivatives of vitamin D_3, 1,24-$(OH)_2D_3$ and 1,24,25$(OH)_3D_3$ stimulated mitotic activity of monocytes as much as calcitriol (fig.3).

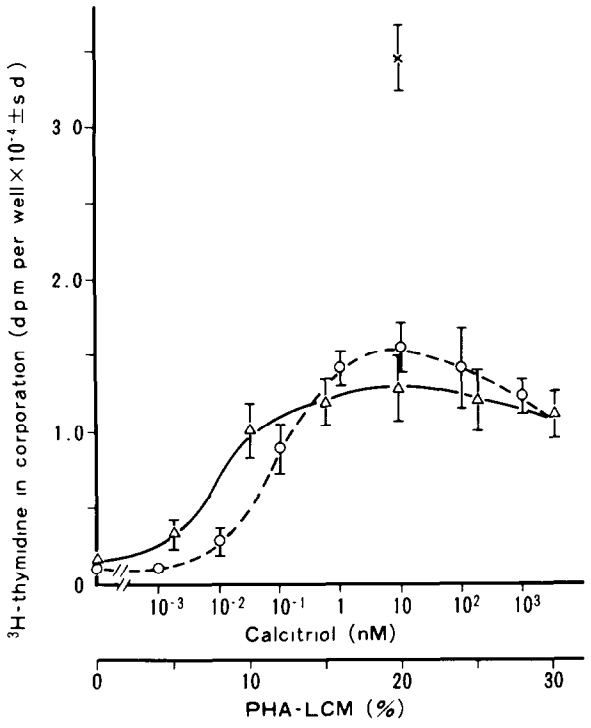

Fig.2. Monocytes were incubated with various concentrations of calcitriol (○), PHA-LCM (△) or both 20% PHA-LCM and 10 nM calcitriol (×). [^{3}H]Thymidine incorporation was measured on the 9th day of culture. Data are means ± SD of triplicate cultures of one experiment.

1(OH)D_3 and 25(OH)D_3 failed to show such activity. These data correlate well with the previous study of Suda and Tanaka [10,11], who examined the binding affinity of vitamin D_3 derivatives for their receptors in myeloid leukemic cell line. They reported that 1,25$(OH)_2D_3$ and 1,24$(OH)_2D_3$ have maximal binding affinity and were most potent in inducing differentiation, followed successively by 1,24,25$(OH)_3D_3$, 25(OH)D_3, 1(OH)D_3, 24,25$(OH)_2D_3$.

Other hormones or chemicals, such as parathyroid hormone, calcitonin, dexamethasone, retinoic acid, testosterone and prostaglandin E_1, failed to promote the growth of monocytes (table 1). It is suggested that the activity to stimulate the proliferation of monocytes in culture may be limited to the biologically active form of vitamin D_3.

The results raise the question as to why it takes 4 days for the calcitriol-treated monocytes to begin proliferation. We thought that this delay may be due to the immaturity of monocytes in early

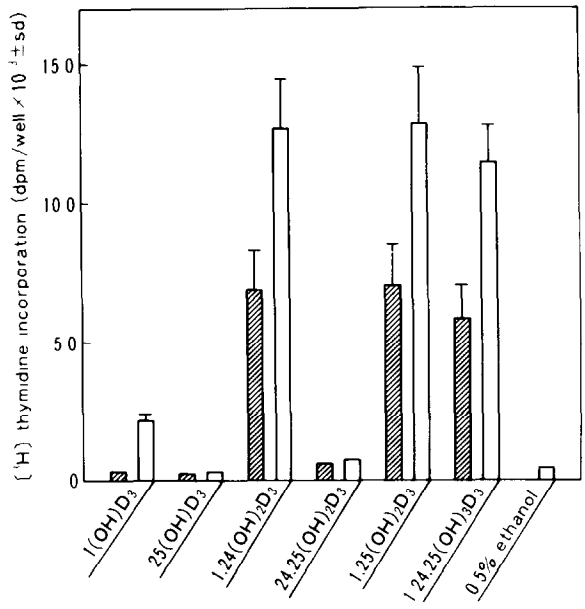

Fig.3. Monocytes were incubated with 0.1 nM (hatched bars) or 10 nM (unhatched bars) of 1,25$(OH)_2D_3$, 1(OH)D_3, 25(OH)D_3, 1,24$(OH)_2D_3$, 24,25$(OH)_2D_3$, 1,24,25$(OH)_3D_3$ or 0.5% ethanol (control). [^{3}H]Thymidine incorporation into monocytes was measured on the 9th day of culture. Data are means ± SD of triplicate cultures of one experiment.

Table 1

Monocyte-growth activity of various hormones or chemicals

Hormone or chemical	Concentration	[^{3}H]Thymidine incorporation (dpm/well ± SD)
Control		517 ± 79
Human parathyroid hormone (1–44)	100 ng/ml	511 ± 129
Human calcitonin	3 μM	317 ± 50
Dexamethasone	0.1 nM	496 ± 79
	10 nM	316 ± 123
Retinoic acid	0.1 nM	571 ± 31
	10 nM	541 ± 69
Calcitriol	10 nM	4891 ± 630
Testosterone	0.1 nM	591 ± 59
	10 nM	498 ± 50
Prostaglandin E_1	0.1 μM	620 ± 142
	10 μM	475 ± 49

Monocytes were incubated with 10% autologous serum. [^{3}H]Thymidine incorporation into monocytes was measured as described in section 2. Each value represents mean ± SD of triplicate cultures of one experiment

culture. This notion led us to examine the effect of calcitriol on monocytes that were incubated for 4 days prior to addition of calcitriol. Within 24 h of incubation, calcitriol-treated monocytes showed significantly more [^{3}H]thymidine uptake than untreated monocytes (fig.1A). The monocytes freshly isolated from peripheral blood may be too immature to proliferate and in vitro maturation of monocytes may be associated with their proliferation.

Another question to arise is why addition of both calcitriol and PHA-LCM had an additive effect on the mitotic activity of monocytes. It is possible that calcitriol may stimulate mitosis of monocytes by a mechanism different from that of lymphocyte-derived MoGF. We thought that calcitriol may stimulate mitosis of monocytes by inducing the secretion of their own MoGF, for preliminary data showed that the MoGF activity was present in calcitriol-treated monocyte-culture medium. The MoGF activity was present even after repeated dialysis of the culture medium for several days (not shown). However, it is difficult to demonstrate the presence of their own MoGF because MoGF activity in the medium may be due to the presence of calcitriol in the serum of the culture medium. Calcitriol is known to be a promoter of differentiation in myeloid leukemic cell clones because calcitriol induces them to differentiate into mature macrophages or phagocytes with a bone resorbing function [12–15]. Moreover, it is demonstrated that calcitriol plays a critical role in the formation of multinucleated giant cells by promoting fusion of macrophages [16]. These results suggest that calcitriol is a promoter of differentiation that induces myeloid precursors into mature phagocytes or osteoclasts. However, the proliferative effect of calcitriol on cells of myelocyte-macrophage lineage has not yet been reported. Our study suggests that calcitriol is, in normal human circulating monocytes, a potent stimulator of proliferation.

ACKNOWLEDGEMENTS

We thank Dr T. Suda, Department of Biochemistry, Showa University, for reading the manuscript and Y. Nishii, Chugai Pharmaceutical Co. Ltd, for providing calcitriol and other vitamin D_3 derivatives.

REFERENCES

[1] Ohta, M., Ozawa, K., Okabe, T., Urabe, A. and Takaku, F. (1985) Biochem. Biophys. Res. Commun., in press.
[2] Wintrobe, M.M., Lee, G.R., Boggs, D.R., Bithell, T.C., Foerster, J., Athens, J.W. and Lukens, J.N. (1981) in: Clinical Hematology, 8th edn, pp.239–255, Lea and Febiger, Philadelphia.
[3] Klebanoff, S.J. (1982) in: Cecil Textbook of Medicine (Wyngaarden, J.B. and Smith, L.H. eds) pp.905–909, Saunders, Philadelphia.
[4] Van Furth, R., Dulk, M.M.C.D.D. and Matie, H. (1973) J. Exp. Med. 138, 1314–1330.
[5] Van Furth, R., Raeburn, J.A. and Van Zwet, T.L. (1979) Blood 54, 485–500.
[6] Ohta, M., Okabe, T., Ozawa, K., Urabe, A. and Takaku, F. (1985) Ann. NY Acad. Sci., in press.
[7] Karpas, A., Hayhoe, F.G.J., Greenberger, J.S., Barker, C.R., Cawley, J.C., Lowenthal, R.M. and Moloney, W.C. (1977) Leukemia Res. 1, 35–49.
[8] Tsutiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T. and Tada, K. (1980) Int. J. Cancer 26, 171–176.

[9] Nakagawara, A. and Nathan, C.F. (1983) J. Immunol. Methods 56, 261–268.
[10] Tanaka, H., Abè, E., Miyaura, C., Kuribayashi, T., Konno, K., Nishii, Y. and Suda, T. (1982) Biochem. J. 204, 713–719.
[11] Suda, T., Shiina, Y., Abe, E., Miyaura, C., Yamada, S., Ohmori, M., Nakayama, K., Takayama, H., Matsunaga, I. and Nishii, Y. (1983) in: Endocrinology (Shizume, K. et al. eds) pp.213–217, International Congress Series 598, Excerpta Medica, Amsterdam, Princeton.
[12] McCarthy, D.M., San Miguel, J.F., Freake, H.C., Green, P.M., Zola, H., Catovsky, D. and Goldman, J.M. (1983) Leukemia Res. 7, 51–55.
[13] Miyaura, C., Abe, E., Kuribayashi, T., Tanaka, H., Konno, K., Nishii, Y. and Suda, T. (1981) Biochem. Biophys. Res. Commun. 102, 937–943.
[14] Dodd, R.C., Cohen, M.S., Newman, S.L. and Gray, T.K. (1983) Proc. Natl. Acad. Sci. USA 80, 7538–7541.
[15] Bar-Shavit, Z., Teitelbaum, S.L., Reitsma, P., Hall, A., Pegg, L.E., Trial, J. and Kahn, A.J. (1983) Proc. Natl. Acad. Sci. USA 80, 5907–5911.
[16] Abe, E., Miyaura, C., Tanaka, H., Shiina, Y., Kuribayashi, T., Suda, S., Nishii, Y., DeLuca, H.F. and Suda, T. (1983) Proc. Natl. Acad. Sci. USA 80, 5583–5587.