Effect of Vitamin D on Humoral and Cell-Mediated Immune Response and Functional Activity of Peritoneal Macrophages

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1,25-Dihydroxyvitamin D_3, an active metabolite of vitamin D, decreases the number of antibody-producing cells in C57Bl/6 mice immunized with sheep erythrocytes, suppresses lymphocytes proliferation in response to stimulation with pokeweed mitogen and concanavalin A, and stimulates functional activity of macrophages.

Key Words: *1,25-dihydroxyvitamin D_3; antibody-forming cells; T lymphocytes; B lymphocytes; peritoneal macrophages*

The discovery of immunoregulatory properties of vitamin D is an important event in the investigation of the biological effects of vitamin D [1,8,11]. This is surprising because over many decades millions children took vitamin D for prophylaxis and therapy of rickets and various vitamin D-dependent diseases. However, most of these studies were performed *in vitro*, and their results may not coincide with the results of *in vivo* experiments. Therefore, the data obtained *in vivo* are much more valuable for medical practice. In the present study we examined the effect of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), a vitamin D metabolite exhibiting its main biological activities, on the number of cells producing antibodies to sheep erythrocytes (APC), lymphocyte proliferation induced by pokeweed mitogen (PWM) and concanavalin A (ConA), and functional activity of peritoneal macrophages in mice. It has been shown that $1,25(OH)_2D_3$ inhibits proliferation of T and B lymphocytes, suppresses production of some cytokines, and potentiates cyclosporine A-induced immune suppression [8,10,11,14].

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MATERIALS AND METHODS

Experiments were carried out on 60 male C57Bl/6 mice weighing 22-24 g. $1,25(OH)_2D_3$ was administered orally for 5 days in a dose of 100 ng/day. Some mice were then immunized with sheep erythrocytes (0.2 ml of 10% suspension). The number of splenic APC was determined by the method of Jerne. Functional activity of T and B cells was assessed in the blast-transformation response to ConA and PWM, respectively, by measuring the intensity of H^3-thymidine incorporation into DNA. For this purpose, 10^7 lymphocytes were cultured in RPMI-1640 medium supplemented with 10% embryonal calf serum, antibiotics, and mitogens (10 μg) for 72 h at 37°C. Thymidine (1 μCi) was added to each sample 6 h before the end of incubation. The samples were washed several times with RPMI-1640, and the pellet was lysed with 1 ml 10% Triton X-100. Aliquots (0.2 ml) of the lysate were transferred into scintillation vials, and specific radioactivity was counted on an Intertechnique counter. The phagocytic activity of peritoneal macrophages toward *Staphylococcus aureus* (Zhaev strain) was determined in a cell culture [4]. The percentage of phagocytizing macrophages (phagocyte count) and the number of bacteria per phagocyte (phagocytic index) were calculated.

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The data were analyzed using the Student *t* test.

RESULTS

As Fig. 1 shows, vitamin D markedly suppressed APC production and significantly inhibited blastogenesis induced by ConA and PWM. Vitamin D increased both the phagocyte count and phagocytic index.

When analyzing the mechanisms of the observed phenomena, the inhibiting effect of peroxidation products on T and B lymphocytes [8,11] and possible prooxidant properties of vitamin D [13] should be considered. Vitamin D elicits direct toxic effect on the immune system, which was confirmed by our previous investigation of the ability of vitamin D to induce lymphopenia [2]. It was reported that peroxidation products inhibit blast-transformation of T and B lymphocytes [9] and induce aggregation of human γ-globulin [15]. *In vitro* experiments showed that $1,25(OH)_2D_3$ inhibits cytokine production by lymphocytes, particularly that of interleukin-2 [11]. Moreover, we have demonstrated that vitamin D stimulates secretion of immunosuppressive hormones in the adrenals [3]. In addition, $1,25(OH)_2D_3$ reduces the helper/suppressor ratio [7]. All these mechanisms may to various extent contribute to the phenomenon observed in this study. Concerning the enhanced phagocytic activity of macrophages, it should be noted that some prooxidants (for example, vitamin D) enhance antibacterial activity of macrophages via the effects of some peroxidation products [12]. Vitamin D also promotes the release of hydrogen peroxide and some lysosomal enzymes from macrophages [5]. Stimulation of phagocytosis is an energy-dependent process; unfortunately, there are no published data on the involvement of vitamin D in this process.

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TABLE 1. Effect of $1,25(OH)_2D_3$ on the Phagocytic Activity of Peritoneal Macrophages from C57Bl/6 Mice

Group	Parameters of phagocytosis	
	phagocyte count, %	phagocytic index
Control (*n*=20)	46.5±1.7	3.6±0.1
Experiment (*n*=20)	72.5±1.5*	5.1±0.1*

Note. Asterisk indicates values significantly different from the control.

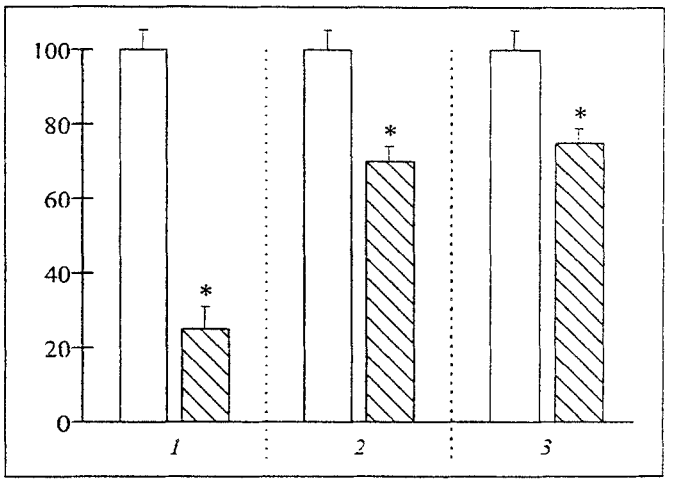

Fig. 1. Effect of $1,25(OH)_2D_3$ on some parameters of humoral and cell-mediated immunity in C57Bl/6 mice. White bars: parameters in the control groups taken as 100%; hatched bars: parameters in $1,25(OH)_2D_3$-treated groups. Effect of $1,25(OH)_2D_3$ on the number of APC (*1*), blastogenic response of lymphocytes stimulated with ConA (2) and PWM (3). Asterisk indicates statistically significant differences in comparison with the control.

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REFERENCES

1. K. D. Pletsityi, V. A. Evseev, I. N. Gazdarova, *et al.*, *Immunologiya*, No. 2, 47-50 (1982).
2. K. D. Pletsityi, G. T. Sukhikh, T. V. Davydova, *et al.*, *Byull. Eksp. Biol. Med.*, **104**, No. 8, 203-204 (1987).
3. K. D. Pletsityi and I. N. Shaternikova, *Pat. Fiziol.*, No. 4, 40-44 (1984).
4. V. P. Selezneva, "The role of cells of the macrophagal system in the antipertussis immunity," Author's Synopsis of Dissertation [in Russian], Moscow (1970).
5. W. L. Gluck and J. B. Weinberg, *J. Leukoc. Biol.*, **42**, 498-503 (1987).
6. S. Gupta, D. Fass, M. Shimizu, *et al.*, *Cell. Immunol.*, **121**, 290-297 (1989).
7. J. M. Lemire, J. S. Adams, V. Kermani-Arab, *et al.*, *J. Immunol.*, **134**, 3032-3035 (1985).
8. S. C. Manolagas, D. M. Provvedini, and S. D. Tsoukas, *Mol. Cell. Endocrinol.*, **43**, 113-122 (1985).
9. Y. Nishida, K. Tanimoto, and I. Akaoka, *Clin. Immunol. Immunopathol.*, **19**, 319-324 (1981).
10. H. Reichel, H. P. Koeffler, A. Tobler, *et al.*, *Proc. Natl. Acad. Sci. USA*, **84**, 3385-3389 (1987).
11. W. F. C. Rigby, *Immunol. Today*, **9**, 768-770 (1988).
12. C. A. Rock, *Am. Rev. Respir. Dis.*, **138**, 768-770 (1988).
13. V. B. Spirichev and N. V. Blazhievich, *Int. J. Vitam. Nutr. Res.*, **39**, 30-35 (1969).
14. C. D. Tsoukas, D. Watry, S. S. Escobar, *et al.*, *J. Clin. Endocrinol. Metab.*, **11**, 650-651 (1989).
15. D. G. Wickens, T. L. Graff, J. Lunec, *et al.*, *Agents Actions*, **11**, 650-651 (1981).