1,25-Dihydroxyvitamin D_3 potentiates the decreased response of lymphocytes from atopic subjects to agents that increase intracellular cyclic adenosine monophosphate

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The inhibitory effect of prostaglandin E_2, histamine, isobutylmethylxanthine, and 1,25-dihydroxyvitamin D_3 (1,25-[OH]$_2D_3$) on the mitogenic stimulation of peripheral blood lymphocytes from normal and atopic subjects was studied. We found that lymphocytes from atopic patients were less susceptible to inhibition by the three agents that elevate intracellular cyclic adenosine monophosphate (cAMP) concentrations and by the active metabolite of vitamin D (inhibition of 27%, 14%, 12%, and 36% for the atopic patients as compared with 40%, 20%, 22%, and 46% for the normal donors, by the four agents, respectively; $p < 0.02$). The inhibitory effect of the cAMP-elevating agents was potentiated by the addition of 1,25-(OH)$_2D_3$ to the lymphocyte cultures. The potentiation was more pronounced on lymphocytes from the atopic donors, increasing their responsiveness to levels comparable to levels of lymphocytes from normal donors. The synthetic corticosteroid, dexamethasone, had a similar potentiating effect on the inhibitory action of prostaglandin E_2. In view of the beneficial action of β-agonists, phosphodiesterase inhibitors, and corticosteroids in the treatment of allergy, the potentiating effect of 1,25-(OH)$_2D_3$ on the action of cAMP-elevating agents may be of therapeutic interest.
(J ALLERGY CLIN IMMUNOL 1990;86:881-5.)

The atopic diathesis is a genetically predisposed, environmentally induced, inflammatory disorder that may be manifested as allergic asthma, allergic rhinitis, or atopic dermatitis.[1] These disorders are prevalent in families, and individuals afflicted with one atopic disorder are more prone to suffer from another atopic disorder at the same or at different periods of their life. Szentivanyi[2] suggested in 1968 that impaired β-adrenergic reactivity may be a primary determinant of atopy. Later, impaired cellular responses to β-adrenergic stimuli, including attenuated increase in cAMP levels, were demonstrated in leukocytes of patients with atopic dermatitis.[3, 4] Subsequent investigations demonstrated that this phenomenon is not limited to β-adrenergic agonists but occurs also with other agents that elevate intracellular cAMP (cAMP promoters) like PGE_1 or histamine.[5, 6] This blunted response may be related to the reduced numbers of receptors for these agents[7-9] or to the increased cAMP phosphodiesterase activity found in leukocytes of atopic patients.[10, 11] Similar changes in the mechanisms that control cAMP levels occur also in states of heterologous desensitization.[12] It is possible that a chronic state of heterologous desensitization is the

Abbreviations used

PGE_1, PGE_2:	Prostaglandin E_1 and E_2
1,25-$(OH)_2D_3$:	1,25-Dihydroxyvitamin D_3
IBMX:	Isobutylmethylxanthine
cAMP:	Cyclic adenosine monophosphate
IL-2:	Interleukin-2

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Supported by United States-Israel Binational Science Foundation Grant No. 85-00276, Jerusalem, Israel.
Received for publication Feb. 13, 1990.
Revised July 23, 1990.
Accepted for publication July 26, 1990.

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1/1/24121

underlying cause for the biochemical changes associated with atopy.[12] The impaired responsiveness to cAMP promoters does not appear to be confined to leukocytes. It has been demonstrated, for instance, that gastric H_2 receptor-mediated function is suppressed in patients with allergic asthma.[13]

1,25-$(OH)_2D_3$, the natural active metabolite of vitamin D and cAMP promoters, inhibit mitogen- and antigen-induced lymphocyte stimulation.[14-17] We have recently demonstrated that 1,25-$(OH)_2D_3$ acts synergistically with agents that increase intracellular cAMP levels to suppress lymphocyte mitogenesis.[18, 19]

Our aim was to study the in vitro effect of 1,25-$(OH)_2D_3$ on the responsiveness of lymphocytes from atopic subjects to the action of cAMP promoters. We have found that mitogen-stimulated lymphocytes from atopic patients are less prone to inhibition by cAMP promoters and by 1,25-$(OH)_2D_3$ than lymphocytes from normal subjects. In agreement with our previous study, we found that 1,25-$(OH)_2D_3$ potentiated the effect of cAMP promoters on lymphocytes from normal donors. This potentiating effect was more pronounced on lymphocytes from atopic subjects. Moreover, in the presence of 1,25-$(OH)_2D_3$, the inhibitory effect of cAMP promoters on cells from atopic subjects was indistinguishable from the effect on cells from normal donors.

MATERIAL AND METHODS

Subjects

Twenty-three subjects (13 male and 10 female subjects) with allergic rhinoconjunctivitis and/or asthma participated in the present study. Subjects' ages ranged from 11 to 56 years. Each subject had at least two positive immediate skin tests and elevated serum IgE levels. None of the subjects had received any oral glucocorticosteroids for 6 months, nor had they received antihistamines or oral bronchodilators (theophylline) for the last 48 hours before blood sampling. Inhaled β-agonists were stopped for at least 24 hours before the experiment. Inhaled beclomethasone dipropionate was taken in a low dose up to 400 μg/day and stopped 48 hours before blood sampling. Fourteen normal volunteers with no personal or family history of atopy constituted the control population. Age and sex distributions were similar to those of the atopic group.

Lymphocyte mitogenesis

Mononuclear cells were prepared from heparinized venous blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density-gradient centrifugation. The cells were suspended in RPMI 1640 medium (Biological Industries, Beth-Haemek, Israel) containing 5% heat-inactivated pooled human AB serum supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were cultured (10^6/ml) in flat-bottomed microtiter plates (Nunc GMB, Roskilde, Denmark) (200 μl per well) in the presence of 1 μg/ml of phytohemagglutinin (purified, Wellcome Laboratories, Beckenham, England) and indomethacin, 5 μg/ml, to prevent possible interference of endogenously produced prostaglandins. The cells were cultured for 72 hours at 37° C in a humidified 5% CO_2 95% air atmosphere. PGE_2, 3×10^{-7} mol/L; histamine, 10^{-5} mol/L; and IBMX, 25 or 100 μmol/L (Sigma Chemical Co., St. Louis, Mo.); 1,25-$(OH)_2D_3$, 10^{-9} mol/L (kindly supplied by Dr. M. Uskokovic, Hoffman-LaRoche, Nutley, N.J.); and dexamethasone, 10^{-8} mol/L, were added at initiation of culture. [^{3}H]Thymidine, 1 μCi per well (2 Ci/mmol, Nuclear Research Center, Negev, Beer-Sheva, Israel), was added at 68 hours of culture. The cells were harvested 4 hours later with an automated cell harvester (Dynatech Corp., Burlington, Maine). Cultures were performed in triplicate. In the presence of phytohemagglutinin and absence of added drugs, thymidine incorporation into mononuclear cells from atopic subjects was 51530 ± 5500 cpm per culture, and from normal subjects, 49120 ± 5910 cpm per culture (means ± SE). Thymidine incorporation into mononuclear cells cultured in absence of mitogen did not exceed 500 cpm per culture.

Calculations and statistical analysis

The extent of inhibition (I) exerted by the various drugs was calculated as follows:

$$I = [1 - (cpm_{+dr}/cpm_{-dr})] \times 100$$

where cpm_{+dr} and cpm_{-dr} denote [^{3}H]thymidine incorporated in presence and absence of drug, respectively. I is expressed as percent inhibition. When the effect of a cAMP promoter was tested in the presence of 1,25-$(OH)_2D_3$ or dexamethasone, its net inhibitory effect was calculated by comparing thymidine incorporation in the culture containing both drugs to that in the culture containing only 1,25-$(OH)_2D_3$ or dexamethasone. Data were analyzed by means of the Student's paired *t* test and the nonparametric sign test comparing normal and atopic subjects assayed at the same time.

RESULTS

The cAMP promoters used in this study increase intracellular levels of cAMP either by a receptor-mediated activation of adenylate cyclase (PGE_2 and histamine) or by inhibition of cAMP phosphodiesterase (IBMX). All three agents had a significantly lower inhibitory effect on lymphocytes from atopic patients than on lymphocytes from normal donors, as judged by both the Student's *t* test and the nonparametric sign test (Table I). It is noteworthy that the inhibitory effect of 1,25-$(OH)_2D_3$, as well, was lower in atopic patients (Table I).

On the basis of our previous findings that 1,25-$(OH)_2D_3$ potentiated the effect of various cAMP promoters on the mitogenic stimulation of normal human lymphocytes,[18, 19] it was of interest to study the

TABLE I. Effects of 1,25-dihydroxyvitamin D_3, dexamethasone, PGE_2, histamine, and IBMX on the mitogenic stimulation of lymphocytes from atopic and normal subjects

cAMP promoter	Steroid hormone	Inhibition (%) (mean ± SEM)			Proportion of atopic subjects responding less than normal subjects*	
		Normal	Atopic			
—	1,25D	46.3 ± 3.2 n = 14	35.6 ± 3.6 n = 22	$p < 0.01$†	16/22	$p < 0.01$‡
PGE_2	—	40.2 ± 3.4	27.1 ± 3.4	$p < 0.01$	19/23	$p < 0.01$
	1,25D	59.9 ± 2.4 n = 14	58.7 ± 3.6 n = 23	NS	9/23	NS
Histamine	—	20.2 ± 2.6	13.6 ± 2.1	$p < 0.01$	13/16	$p = 0.01$
	1,25D	39.7 ± 4.4 n = 10	39.3 ± 3.8 n = 16	NS	10/16	NS
IBMX (25 μmol/L)	—	22.5 ± 3.7	11.6 ± 2.7	$p < 0.02$	14/19	$p < 0.05$
	1,25D	39.3 ± 4.6 n = 13	39.4 ± 4.5 n = 19,20§	NS	10/20	NS
IBMX (100 μmol/L)	—	47.3 ± 4.8	30.4 ± 3.3	$p < 0.02$	16/21	$p = 0.01$
	1,25D	63.0 ± 3.7 n = 13	61.4 ± 3.2 n = 21	NS	10/21	NS
PGE_2	—	36.7 ± 3.3	22.7 ± 5.3	$p < 0.02$	9/10	$p = 0.01$
	DEX	52.8 ± 10.1 n = 6	48.9 ± 5.4 n = 10	NS	6/10	NS

1,25D, 1,25-$(OH)_2D_3$; *NS*, not significant; *DEX*, dexamethasone.
PGE_2 (3×10^{-7} mol/L), histamine (10^{-5} mol/L), IBMX, 1,25D (10^{-9} mol/L) and DEX (10^{-8} mol/L) were added at initiation of cultures.
*Proportion of atopic subjects with lymphocytes less responsive to the inhibitor than lymphocytes from normal control subjects examined at the same time.
†Student's paired *t* test analysis of atopic versus normal subjects examined at the same time.
‡Significance of deviation from random distribution estimated by the nonparametric sign test.
§Number of subjects was 19 for the normal population and 20 for the atopic population.

combined effect of 1,25-$(OH)_2D_3$ and cAMP promoters on lymphocytes from atopic donors. When 1,25-$(OH)_2D_3$ was added to cultures together with each of the cAMP promoters, a synergistic interaction, similar to interaction observed in normal lymphocytes, was found. Moreover, the potentiating effect of 1,25-$(OH)_2D_3$ on the effect of cAMP promoters is twofold to threefold higher on lymphocytes from atopic patients than on lymphocytes from normal donors: an increase of 120% in the effect of PGE_2, 190% in the effect of histamine, and 240% in the effect of IBMX (25 μmol/L on atopic lymphocytes ($p < 0.001$, Student's paired *t* test), as compared to an increase of 50%, 100%, and 70%, respectively, in the inhibitory effects on normal lymphocytes ($p < 0.01$) (Table I). In the presence of 1,25-$(OH)_2D_3$, there was no statistically significant difference between the effects of the cAMP promoters on lymphocytes from the atopic and normal subjects (Table I).

Glucocorticosteroids are widely used in the treatment of atopic disorders. It was of interest to examine if these hormones can also modulate the effect of cAMP promoters on lymphocyte mitogenesis. We found that the glucocorticoid, dexamethasone, potentiated the inhibitory effect of PGE_2 on lymphocytes from atopic and normal subjects (an increase of 115%, $p < 0.02$, Student's paired *t* test, for atopic donors and an increase of 44%, $p < 0.05$, for normal donors) (Table I). Similarly to 1,25-$(OH)_2D_3$, dexamethasone eliminated the difference in sensitivity to PGE_2 between lymphocytes from atopic and normal subjects.

DISCUSSION

We demonstrated in this study that the cAMP promoters, PGE_2, histamine, and IBMX, inhibit the mitogenesis of lymphocytes from atopic donors to a lesser extent than the mitogenesis of lymphocytes from normal donors. This finding is not unexpected in view of the studies of impaired responsiveness to cAMP promoters observed in various target cells, including lymphocytes, from atopic subjects.[2-6] Previous stud-

ies, in smaller donor populations[20] or unpaired experimental design,[21] have not been able to demonstrate a reduced inhibitory effect of PGE_1 or histamine on mitogen-induced DNA synthesis,[20, 21] although Rocklin et al.[20] found such a difference in the effect of PGE_1 on mitogen-induced protein synthesis. To our knowledge, impaired responsiveness to the phosphodiesterase inhibitor, IBMX, has not been reported to date for cells of atopic subjects. The difference in susceptibility between atopic and normal lymphocytes to IBMX was at least as pronounced as the difference found for the receptor-dependent cAMP promoters, PGE_2 and histamine. It appears therefore that lower PGE and histamine-receptor content cannot fully account for the impaired responsiveness of lymphocytes from atopic subjects. Another finding of this study is that the inhibitory effect of $1,25\text{-}(OH)_2D_3$ is less pronounced in atopic patients as compared to normal donors. There is accumulating evidence that $1,25\text{-}(OH)_2D_3$ may have a role in limiting the immune response to offending antigens.[16] It is tempting to suggest that the impaired responsiveness to $1,25\text{-}(OH)_2D_3$ may be involved in the pathophysiology of atopic disorders.

Since the antimitogenic activities of $1,25\text{-}(OH)_2D_3$ and the cAMP promoters are mainly due to inhibition of the production of the lymphocyte growth factor, IL-2,[16, 17] it could be argued that the low responsiveness of lymphocytes from the atopic donors to these agents is due to their greater potential for IL-2 production. That this is not the case may be concluded from a recent study documenting that IL-2 levels in cultures of activated lymphocytes from atopic patients are even lower than in cultures from normal donors.[22] In our view, the most interesting finding of this study is that $1,25\text{-}(OH)_2D_3$ potentiates the effect of cAMP promoters on lymphocytes from atopic donors to a greater extent than on lymphocytes from normal subjects and that this preferential action leads to the disappearance of the difference between the two groups. Since the impaired responsiveness to cAMP promoters, associated with atopy, has been attributed to attenuated increase in cAMP levels, it is possible that $1,25\text{-}(OH)_2D_3$ exerts its action by increasing intracellular cAMP concentrations. This is probably not the case because Rubin and Catherwood[23] have demonstrated that $1,25\text{-}(OH)_2D_3$ decreases, rather than increases, intracellular levels of cAMP in activated lymphocytes. Therefore, $1,25\text{-}(OH)_2D_3$ compensates for, but probably does not correct, the defect in the lymphocytes from atopic donors.

Additional new findings of this study are that dexamethasone interacts synergistically with PGE_2 to inhibit lymphocyte mitogenesis and that, in the presence of dexamethasone, the responsiveness of lymphocytes from atopic and normal donors to PGE_2 is the same. This finding is compatible with numerous studies of synergism between corticosteroids and various cAMP promoters in leukocytes.[24] The synergism between the synthetic glucocorticosteroid, dexamethasone, and the inflammatory agent, PGE_2, may underlie some of its pharmacologic effects in controlling the cellular component of inflammation. Since corticosteroids have a long proven efficacy in the treatment of allergic disorders, the finding that $1,25\text{-}(OH)_2D_3$ has the same effect as dexamethasone in this experimental system may be of interest.

In addition to its role in inflammatory processes, the lymphocyte may serve as a target organ model for the inaccessible cells that are involved in the allergic reaction. This is plausible in view of the evidence that the atopic state has a genetic predisposition[1, 25] and that various cells and organs of atopic subjects share the same defects in their response to cAMP agonists.[13] It is possible therefore that the potentiating effect of $1,25\text{-}(OH)_2D_3$ on the cAMP response system in lymphocytes may be exerted on other target organs that are involved in the pathophysiology of atopic disorders.

In view of the beneficial action of β-agonists and phosphodiesterase inhibitors in the treatment of allergy, the potentiating effect of $1,25\text{-}(OH)_2D_3$ may have some therapeutic implications.

We thank Dr. M. Modan for her advice in the statistical analysis, and Dr. Z. Keinan for his help in this project.

REFERENCES

1. Smith JM. Epidemiology and natural history of asthma, allergic rhinitis, and atopic dermatitis (Eczema). In: Middleton E Jr, Reed CE, Ellis EF, eds. Allergy: principles and practice. St. Louis: CV Mosby, 1978:633-58.
2. Szentivanyi A. The beta-adrenergic theory of the atopic abnormality in bronchial asthma. J ALLERGY 1968;42:203-32.
3. Reed CE, Busse WW, Lee TP. Adrenergic mechanisms and the adenylate cyclase system in atopic dermatitis. J Invest Dermatol 1976;67:333-8.
4. Parker CW, Kennedy S, Eisen AZ. Leukocyte and lymphocyte cyclic AMP response in atopic eczema. J Invest Dermatol 1977; 68:302-6.
5. Busse WW, Lantis SOH. Decreased H_2 histamine granulocyte response in active atopic eczema. J Invest Dermatol 1979; 73:184-7.
6. Archer CB, Morley J, MacDonald DM. Impaired lymphocyte cyclic adenosine monophosphate responses in atopic eczema. Br J Dermatol 1983;109:559-64.
7. Beer DJ, Osband ME, McCaffney RP, Soter NA, Rocklin RE. Abnormal histamine-induced suppressor cell function in atopic subjects. N Engl J Med 1982;306:454-8.
8. Rocklin RE, Thistle L. Reduced prostaglandin E_2 (PGE_2) receptors on atopic T-lymphocytes. Cell Immunol 1986;99: 294-9.

9. Szentivanyi A, Heim O, Schulz P, Szentivanyi J. Adrenoreceptor binding studies with ^{3}H(dihydroalprenolol) and ^{3}H(dihydroergocryptine) on membranes of lymphocytes from patients with atopic disease. Acta Derm Venereol (Stockh) 1980;92(suppl):19-21.
10. Grewe S, Chan SC, Hanifin JM. Elevated leukocyte cyclic AMP phosphodiesterase in atopic disease: a possible mechanism for cyclic AMP agonist hyporesponsiveness. J ALLERGY CLIN IMMUNOL 1982;70:452-7.
11. Butler JM, Chan SC, Stevens S, Hanifin JM. Increased leukocyte histamine release with elevated cyclic AMP-phosphodiesterase activity in atopic dermatitis. J ALLERGY CLIN IMMUNOL 1983;71:490-7.
12. Safko MJ, Chan SC, Cooper KD, Hanifin JM. Heterologeous desensitization of leukocytes: a possible mechanism of beta adrenergic blockade in atopic dermatitis. J ALLERGY CLIN IMMUNOL 1981;68:218-25.
13. Gonzales H, Ahmed T. Suppression of gastric H_2-receptor mediated function in patients with bronchial asthma and ragweed allergy. Chest 1986;89:491-6.
14. Tsoukas CD, Provvedini DM, Manolagas SC. 1,25-dihydroxyvitamin D_3: a novel immunoregulatory hormone. Science 1984;224:1438-40.
15. Koren R, Ravid A, Liberman UA, Hochberg Z, Weisman Y, Novogrodsky A. Defective binding and function of 1,25-dihydroxyvitamin D_3 receptors in peripheral mononuclear cells of patients with end organ resistance to 1,25-dihydroxyvitamin D. J Clin Invest 1985;76:2012-5.
16. Rigby WFC. The immunobiology of vitamin D. Immunol Today 1988;9:54-8.
17. Kammer GM. The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response. Immunol Today 1988;9:222-9.
18. Koren R, Ravid A, Rotem C, Shohami E, Liberman UA, Novogrodsky A. 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. FEBS Lett 1986;205:113-6.
19. Ravid A, Koren R, Narinsky R, Rotem C, Novogrodsky A, Liberman UA. 1,25-Dihydroxyvitamin D_3 and agents that increase intracellular adenosine 3′,5′-monophosphate synergistically inhibit the mitogenic stimulation of human lymphocytes. J Clin Endocrinol Metab 1990;70:1687-92.
20. Rocklin RE, Thistle L, Audera C. Decreased sensitivity of atopic mononuclear cells to prostaglandin E_2 (PGE_2) and prostaglandin D_2 (PGD_2). J Immunol 1985;135:2033-9.
21. Wang SR, Zweiman B. Histamine suppression of human lymphocyte response to mitogen. Cell Immunol 1978;36:28-36.
22. Frajman M, Gonzalez L, Alvarado A, Yock J. Cellular immunity and IgE levels in atopic patients. Allergy 1987;42: 81-4.
23. Rubin JE, Catherwood BD. 1,25-dihydroxyvitamin D attenuates cAMP accumulation in cultured human lymphocytes. Mol Cell Endocrinol 1987;52:213-8.
24. Kaliner M. Mechanisms of glucocorticosteroid action in bronchial asthma. J ALLERGY CLIN IMMUNOL 1985;76:321-9.
25. McMillan JC, Heskel NS, Hanifin JM. Cyclic AMP-phosphodiesterase activity and histamine release in cord-blood leukocyte preparations. Acta Derm Venereol (Stockh) 1985;114(suppl):24-32.