Journal of Cellular Biochemistry 49:26–31 (1992)

Immunomodulatory Role of 1,25-Dihydroxyvitamin D_3

Jacques M. Lemire

Department of Pediatrics, University of California, San Diego, La Jolla, California 92093

Abstract The active vitamin D metabolite 1,25-dihydroxyvitamin D_3 [1,25-D_3] is thought to promote many of its actions through interaction with a specific intracellular receptor. The discovery of such receptors in monocytes and activated lymphocytes has led investigators to evaluate the role of the hormone on the immune system. The sterol inhibits lymphocyte proliferation and immunoglobulin production in a dose-dependent fashion. At a molecular level, 1,25-D_3 inhibits the accumulation of mRNA for IL-2, IFN-γ, and GM-CSF. At a cellular level, the hormone interferes with T helper cell (T_h) function, reducing T_h-induction of immunoglobulin production by B cells and inhibiting the passive transfer of cellular immunity by T_h-clones in vivo. The sterol promotes suppressor cell activity and inhibits the generation of cytotoxic and NK cells. Class II antigen expression on lymphocytes and monocytes is also affected by the hormone.

When given in vivo, 1,25-D_3 has been particularly effective in the prevention of autoimmune diseases such as experimental autoimmune encephalomyelitis and murine lupus but its efficacy has been limited by its hypercalcemic effect. Synthetic vitamin D_3 analogues showing excellent 1,25-D_3-receptor binding but less pronounced hypercalcemic effects in vivo have recently enhanced the immunosuppressive properties of the hormone in autoimmunity and transplantation.

Key words: 1,25-D_3, lymphocytes, immunosuppression, autoimmunity, transplantation

The classic role of the steroid hormone 1,25-dihydroxyvitamin D_3 [1,25-D_3] on calcium homeostasis has long been recognized. The sterol interacts stereospecifically with an intracellular receptor protein leading to gene-regulating functions mediating various biologic responses. The discovery of such receptors in various tissues has contributed to previously unsuspected actions for the hormone. More than a decade ago, specific receptors for the sterol (VDR) were discovered in neoplastic cell lines [1]; soon, it was found that the proliferation of such cancer lines could not only be reduced in the presence of 1,25-D_3 but that these cells could acquire the characteristics of normal monocytes in vitro, leading to the first observations of anti-proliferative and pro-differentiating effects of the active metabolite [2]. Shortly thereafter, the VDR was identified in normal human peripheral blood mononuclear cells, monocytes and lymphocytes, the latter upon activation [3,4]. These findings were the starting point for the study of the potential immunomodulatory role of the hormone. Over the years, various cellular and molecular actions of 1,25-D_3 on the immune system have been described in vitro, but it is only recently that a potential application for the compound in vivo has been evaluated. A review of the properties of 1,25-D_3 on immune functions in vitro will precede the summary of our current knowledge of the immunosuppressive actions of the sterol in vivo.

1,25-DIHYDROXYVITAMIN D_3 INTERACTION WITH CELLULAR IMMUNE FUNCTIONS IN VITRO

Lymphocyte Proliferation

As early as 1984, we [5] and others [6–8] first demonstrated that mitogen activation of lymphocyte proliferation was inhibited in the presence of 1,25-D_3 with maximal activity observed at 10^{-8} M hormone concentration. Subsequent studies revealed that 1,25-D_3-inhibition of T lymphocyte proliferation was associated with the prevention of cells to progress from the early G_1 to the late G_1 phase of the cell cycle [9]. At a molecular level, 1,25-D_3 suppressed mRNA levels for IL-2 [10], interferon-γ [11] and GM-CSF [12]. While addition of IL-2 reversed some of the anti-proliferative effects of the hormone, exoge-

Received January 23, 1992; accepted January 24, 1992.

Address reprint requests to Jacques M. Lemire, Department of Pediatrics, University of California, La Jolla, CA 92093.

nous IL-2 could not reverse the inhibition of IFN-Y production or transferrin receptor expression in the presence of 1,25-D_3 despite cell entry into late G_1 and proliferation, suggesting an immunosuppressive effect dissociable from its anti-proliferative effect [13]. Fractionation of T cells in to T helper cells (T_h) and T suppressor cells (T_s) revealed that, despite little difference in VDR expression between T_h and T_s [14], 1,25-D_3 inhibited the proliferation of T_h but not T_s cells [15,16]. Activation signals and cell culture conditions have also influenced the antiproliferative effect of 1,25-D_3 [17].

Helper Function

Pre-incubation of T_h with 1,25-D_3 before coculture experiments with B and T_s revealed that 1,25-D_3 abrogated the inducing effect of T_h on Ig synthesis by B cells [15]. Exogenous IL-2 could restore the T_h-directed Ig production by B cells [18]. Additional evidence for a 1,25-D_3-mediated inhibition of T_h was provided by the ability of the sterol to inhibit a biological activity of an autoimmune T_h clone, delayed-type hypersensitivity (DTH) (see Autoimmunity). By its ability to inhibit IL-2, IFN-γ, and the transfer of DTH, a pattern characteristic of T_h type 1 [19], the sterol could be selecting for this T helper subtype.

Suppressor Function

The hormone appears to exert a "permissive" or even enhancing effect on suppressor activity. In co-cultures of B cells with T cell subsets, 1,25-D_3 did not reverse the suppression provided by T_s on T_h-mediated Ig production by B cells [15]. More evidence for a role of T_s on the 1,25-D_3-inhibition of Ig production of unfractionated lymphocytes was suggested by the fact that only after T_s were removed from the 1,25-D_3-treated lymphocytes that restoration of Ig production was observed with exogenous IL-2 [18].

In recent experiments, using the in vitro model of transplant compatibility, the mixed lymphocyte reaction (MLR), the addition of 1,25-D_3 to a primary MLR contributed to a significant enhancement of suppressor cell activity [20]. Effector cells from a primary MLR incubated with 10^{-8} M 1,25-D_3 displayed up to 67% suppression of a fresh autologous MLR, an effect similar in magnitude to that of cyclosporine [21].

Cytotoxic Function

Cytotoxicity of NK and T cytotoxic cells can be regulated by 1,25-D_3. Merino et al. [22] have shown that 1,25-D_3, in a time- and dose-dependent fashion, could inhibit the generation of cytotoxic activity from cultured CD16+ peripheral blood NK cells. The sterol, however, was unable to interfere with the cytotoxic function of NK cells already established, suggesting an inhibitory effect at the activation level. In the human MLR, we also found that 1,25-D_3 could significantly inhibit the generation of cytotoxic cells [20]. For both studies described, incubation of target cells with 1,25-D_3 did not modify the lytic activity, suggesting that the hormone does not directly alter NK or T-cytotoxic cells effector functions, but rather interferes with the activation or generation of such cells.

B Cells

In addition to the inhibition of Ig production by B cells mediated by T_h, a direct effect of the sterol on B cell Ig production has been suggested using purified B cells [23] or B cells immortalized by Ebstein-Barr virus [24]. However, further indirect inhibition of B cell function could be mediated by monocytes/macrophages through IL-1 [25]. At this time, the effect of 1,25-D_3 on IL-4, a B-cell differentiating factor, has yet to be characterized.

Antigen Expression and Monocyte/Macrophage Function

In addition to the action of the sterol on cellular function, 1,25-D_3 may exert additional immunomodulatory properties by affecting class-II antigen expression on cells of immune lineage. Monocytes/macrophages constitutively express MHC class II antigens (HLA-DR in humans, Ia in mice). Initial observations using a murine monocyte/macrophage tumor cell line, WEHI-3, suggested an enhancement of IFN-γ-induced Ia expression [26]. However other investigators have shown an opposite effect [27]. Recent studies have suggested that 1,25-D_3 could reduce HLA-DR and CD4+ expression by monocytes while not affecting class I antigen expression [28]. Our recent observations also revealed a reduction of class II antigen expression on lymphocytes from the MLR incubated with 1,25-D_3 with no reduction in class I antigen expression [20]. Class II antigen expression was also reduced in a melanoma cell line incubated

in the presence of 1,25-D_3 [29]. Interestingly, the active metabolite could also inhibit the ability of IFN-γ to induce class II antigen expression on non "immune" cells such as non-transformed rat thyroid follicular epithelial cells and mouse testicular Leydig cells [30]. Enhancement of class II antigen expression is a common feature of autoimmunity and often precedes the onset of disease [31]. These observations suggest that 1,25-D_3, directly or through IFN-γ, could not only reduce the antigenicity of cells of the immune system but could also reduce tissue antigenicity. The latter could then reduce the amplification of the immune response seen in various autoimmune disorders.

Finally, the effect of 1,25-D_3 on normal monocyte/macrophage function remains controversial. In addition to the effect of the hormone on class II antigen expression mentioned, monocytes incubated in the presence of 1,25-D_3 can increase [27,32,33] or decrease [34] IL-1 secretion. The sterol can also preserve adherence and normal protein synthesis by monocytes during physiological stress [35] and can enhance intracellular killing of organisms [36]. Impaired phagocytic function of peritoneal macrophages of vitamin D deficient mice could be corrected by the administration of 1,25-D_3 in vitro and in vivo [37]. These observations suggest that, in contrast to its immunosuppressive effect on lymphocytes, 1,25-D_3 may enhance monocyte/macrophage function.

Despite the discovery of VDR in almost all systems studied [38] and the effect of the hormone on immune cells in vitro, an immunomodulatory role for the hormone in pathologic conditions has yet to be defined. Contrary to the serum calcium elevating effect directly attributed to 1,25-D_3 in sarcoidosis [39], lymphoma [38], and anephric patients with end-stage renal disease [40], no systemic immunosuppression has been described in disease states so far. At best, it was suggested that higher concentrations of 1,25-D_3 than circulating levels found at sites of inflammation may act in an autocrine/paracrine fashion to modulate local lymphocyte function [17,27,41].

Of interest, the immunoregulatory properties of 1,25-D_3 in vitro appear to be similar, in many respects, to cyclosporine [21]. Both compounds act preferentially on T lymphocytes during initial activation by antigen. They both appear to select the T helper cell by inhibiting lymphokine production at a genomic level. The generation of cytotoxic and NK cells are both inhibited in the presence of both 1,25-D_3 and cyclosporine, but neither seems to interfere with the function of such cells once generated. Finally, they both allow for enhancement or "sparing" of suppressor function, a key element in the efficacy of cyclosporine in tolerance of transplantation.

IMMUNOSUPPRESSIVE PROPERTIES OF 1,25-D_3 IN VIVO (FIG. 1)

In recent years, new therapeutic modalities have been proposed for the sterol. The availability of synthetic 1,25-D_3 analogues with similar properties to the natural hormone but with less hypercalcemic effects have renewed the search for potential application for 1,25-D_3 in vivo. As shown in the figure, the pro-differentiating effect of the metabolite has led to administer the hormone to mice bearing lymphoproliferative neoplasms. The hormone could prolong survival of mice receiving injections of leukemic cell lines and exert an antimetastatic effect [42]. The antiproliferative effect of the metabolite has contributed to the first recognized human application of non-phosphocalcemic actions of 1,25-D_3 in psoriasis (refer to Kragballe's Prospect article in this issue). Finally and most recently, the immunosuppressive effect of 1,25-D_3 has been studied in animal models of autoimmunity and transplantation.

AUTOIMMUNITY

Three murine models of experimental autoimmunity have been studied so far for the immunomodulatory properties of 1,25-D_3: experimental autoimmune encephalomyelitis (model of human multiple sclerosis), experimental systemic lupus erythematosus, and experimental autoimmune thyroiditis.

In experimental autoimmune encephalomyelitis (EAE), a clinical paralysis can be induced in susceptible animals within 12 to 15 days by the injection of a nervous tissue antigen (myelin basic protein [MBP]) in adjuvant. If the animals survive, a rise in antibody titer to MBP will be present within 2 to 4 weeks after immunization. We found that the administration of 0.1 μg 1,25-D_3 intraperitoneally (I.P.) every other day for 15 days, starting three days prior to immunization, significantly prevented the development of EAE [43]. The rise in antibody titer to MBP was also abrogated. Treatment with the hormone inhibited histologic lesions of EAE. In this

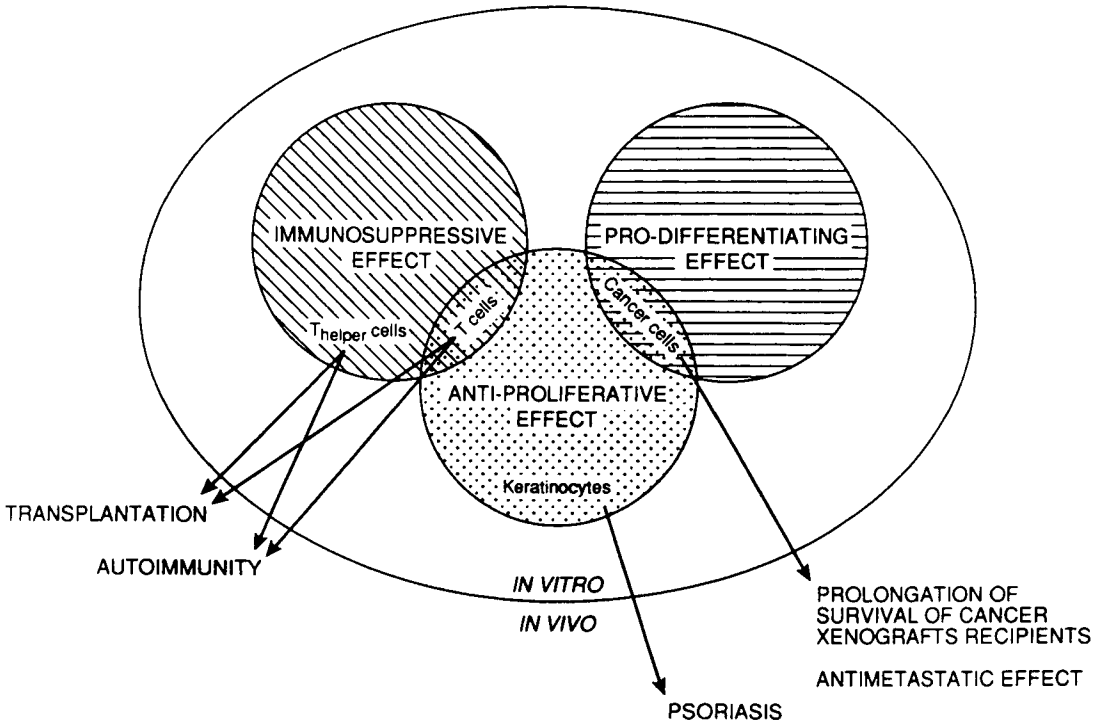

Fig. 1. The three principal non-classical actions of 1,25-D_3 in vitro and their therapeutic applications in vivo.

principally cell-mediated model of autoimmunity, the T_h lymphocyte plays a key role. Clones of MBP-specific T_h isolated from sensitized animals and cultured in vitro exert various biological functions mediated in vivo, including transfer of disease and delayed-type hypersensitivity (DTH) [44]. Using such T_h clones, we recently found that these clones expressed the VDR [45]. Upon exposure to $\geq 10^{-8}$M 1,25-D_3 during incubation with MBP and prior to transfer, the T_h cell clones lost their ability to elicit a DTH response in the recipient in vivo. Of interest, this inhibitory effect on passive transfer of DTH was seen in the absence of significant inhibition of T_h clones proliferation, a dissociation of immunosuppressive effect from antiproliferative action of the hormone as previously suggested in vitro [13].

In experimental lupus, the effect of 1,25-D_3 and related analogues has been studied in MRL/l mice. These autoimmune mice spontaneously develop many of the human manifestations of lupus. A polyclonal T_h cell stimulation of B cell activity has been described in those mice and could contribute to the pathogenesis of the disease. From 4 weeks of age, 1,25-D_3, 0.1 μg I.P. every other day, was extremely effective in the prevention of lupus-associated skin lesions [46]. A reduction of proteinuria and autoantibody titers was also observed in 1,25-D_3-treated animals. The oral administration of a synthetic vitamin D analogue, 22-oxa-1,25-D_3, up to 100 ng/kg 5 days a week from 6 weeks of age until death prolonged survival, reduced proteinuria, and improved histopathological manifestations of the disease [47]. A normalization of lymphocyte phenotypes in thymus and spleen was also observed in treated animals. Another vitamin D_3 analogue, 1,24R-D_3, given at 0.1 μg/kg/day, I.P., 5 days a week, from 6 weeks of age, was also effective in preventing proteinuria and normalizing thymic lymphocyte phenotypes [48].

In experimental autoimmune thyroiditis, manifested histologically by cellular thyroid infiltration and anti-thyroglobulin antibody production, the administration of up to 0.2 μg/kg/day 1,25-D_3, intragastrically, from the day of immunization and daily for 21 days, did not affect the incidence of thyroiditis and only reduced by up to 26% the severity of histological lesions [49]. The effect of cyclosporine was identical to the one observed with 1,25-D_3 when used alone. However, the addition of cyclosporine to 1,25-D_3 produced a lower incidence of thyroid pathology and a significantly milder disease compared to controls.

In all autoimmune models analyzed, 1,25-D_3 was effective, alone or with cyclosporine, in suppressing clinical or paraclinical expressions of disease when administered prior to disease induction or at an early age prior to disease expression.

TRANSPLANTATION

The active vitamin D metabolite was effective in prolonging rat cardiac allograft survival or skin graft survival in mice [50]. However, the doses of 1,25-D_3 required to produce adequate immunosuppression were associated with severe toxicity in the recipients and reduced survival. A recent study of a synthetic 1,25-D_3 analogue, 1,25-Δ^{16}-D_3 (Hoffmann-La Roche, Nutley, NJ), exhibiting more potent suppressive effect than 1,25-D_3 in vitro and less hypercalcemia in vivo, was conducted in mice. Hearts from C3H mice were transplanted into histoincompatible Balb/c mice [51]. The administration of 1,25-D_3, 0.1 μg I.P., starting 3 days prior to transplantation and every other day until rejection (disappearance of visual observation of heart beat), had no significant effect on graft survival; higher doses of the hormone were toxic. However, 0.2 μg 1,25-Δ^{16}-D_3, given every other day, significantly prolonged graft survival; mean survival time was 27.2 ± 4 days compared to 11.57 ± 0.5 days for controls.

PROSPECTS

The evidence so far suggests that the hormone 1,25-D_3 exerts immunomodulatory properties in vitro and in vivo. Investigations of mechanisms of action of the metabolite at a cellular and molecular level should further clarify the interaction of the hormone with the immune system. What is the interaction between VDR, T cell receptor, and activation signals [52]? Can 1,25-D_3 exert immunomodulatory properties at a non-genomic level like its very rapid effects on calcium transport, transcaltachia?

While those questions and others are being addressed, therapeutic applications for the hormone are being studied. The non-hypercalcemic 1,25-D_3 analogues will definitely provide an opportunity to enhance the immunosuppressive effects of the hormone but without its toxicity. Moreover, the synergistic effect observed in the presence of cyclosporine will potentially lead to the use of a synthetic 1,25-D_3 analogue in the treatment of autoimmune diseases or in transplantation, alone or in combination, in order to reduce toxicity of both compounds.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health, grant R29-DK-39024, and by the Children's Fund Inc., Houston, TX. The author thanks John S. Adams, M.D., for review of the manuscript and helpful suggestions and Ms Ronda Bollinger for excellent secretarial assistance.

REFERENCES

1. Eisman JA, Martin TJ, MacIntyre I, Moseley JM: Lancet 2:1335–1336, 1979.
2. Abe E, Miyawra C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiki S, Suda T: Proc Natl Acad Sci USA 78:4990–4994, 1981.
3. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC: Science 221:1181–1183, 1983.
4. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM: J Clin Endocrinol Metab 57:1308–1310, 1983.
5. Lemire JM, Adams JS, Sakai R, Jordan SC: J Clin Invest 74:657–661, 1984.
6. Tsoukas CD, Provvedini DM, Manolagas SC: Science 224:1438–1440, 1984.
7. Rigby WFC, Stacy T, Fanger MW: J Clin Invest 74:1451–1455, 1984.
8. Bhalla AK, Amento EP, Serog B, Glimcher LH: J Immunol 133:1748–1754, 1984.
9. Rigby WFC, Noelle RJ, Krause K, Fanger MW: J Immunol 135:2279–2286, 1985.
10. Rigby WFC, Denome S, Fanger MW: J Clin Invest 79:1659–1664, 1987.
11. Reichel H, Koeffler HP, Tobler A, Norman AW: Proc Natl Acad Sci USA 84:3387–3389, 1987.
12. Tobler A, Gasson J, Reichel H, Norman AW, Koeffler HP: J Clin Invest 79:1700–1705, 1987.
13. Rigby WFC, Hamilton BJ, Waugh MG: Cell Immunol 125:396–414, 1990.
14. Provvedini DM, Manolagas SC: J Clin Endocrinol Metab 68:774–779, 1989.
15. Lemire JM, Adams JS, Kermani-Arab V, Bakke A, Sakai R, Jordan SC: J Immunol 34:3032–3035, 1985.
16. Vanham G, Ceuppens JL, Bouillon R: Cell Immunol 124:320–333, 1989.
17. Manolagas SC, Hustmyer FG, Yu X-P: Kidney Int 38:S9–S16, 1990.
18. Jordan SC, Lemire JM, Sakai R, Toyoda M, Adams JS: Mol Immunol 27:95–100, 1990.
19. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: J Immunol 136:2348–2357, 1986.
20. Meehan MA, Kerman RH, Lemire JM: Cell Immunol (in press).
21. Hess AD, Colombani PM, Esa A: In Williams GM, et al. (eds): "Kidney Transplantation. Diagnosis and Treatment." New York: Marcel Dekker Inc., 1986, pp 353–382.
22. Merino F, Alvarez-Mon M, De La Hera A, Ales JE, Bonilla F, Durantez A: Cell Immunol 118:328–336, 1989.

23. Iho S, Takahashi T, Kura F, Sugiyama H, Hoshino T: J Immunol 236:4427–4431, 1986.
24. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC: J Immunol 136:2734–2740, 1986.
25. Chen W-C, Vayuvegula B, Gupta S: Clin Exp Immunol 69:639–646, 1987.
26. Morel PA, Manolagas SC, Provvedini DM, Wegmann DR, Chiller JM: J Immunol 136:2181–2186, 1986.
27. Amento EP: Steroids 49:55–72, 1987.
28. Rigby WFC, Waugh M, Graziano RF: Blood 76:189–197, 1990.
29. Carrington MN, Tharp-Hiltbold B, Knoth J, Ward FE: J Immunol 140:4013–4018, 1988.
30. Tokuda N, Mano T, Levy RB: Endocrinology 127:1419–1427, 1990.
31. Wuthrich RP, Yui MA, Mazoujian G, Nabavi N, Glimcher LH, Kelly VE: Am J Pathol 134:45–51, 1989.
32. Bhalla AK, Amento EP, Krane SM: Cell Immunol 98: 311–322, 1986.
33. Fagan DL, Prehn JL, Adams JS, Jordan SC: Mol Immunol 5:179–186, 1991.
34. Tsoukas CD, Watry D, Escobar SS, Provvedini DM, Dinarello CA, Hustmyer FG, Manolagas SC: J Clin Endocrinol Metab 69:127–133, 1989.
35. Polla BS, Healy AM, Amento EP, Krane SM: J Clin Invest 77:1332–1339, 1986.
36. Crowle AJ, Ross EJ, May MH: Infect Immun 55:2945–2950, 1987.
37. Bar-Shavit Z, Noff D, Edelstein S, Meyer M, Shibolet S, Goldman R: Calcif Tissue Int 33:673–676, 1981.
38. Reichel H, Koeffler HP, Norman AW: N Engl J Med 320:980–991, 1989.
39. Bell NH, Stern PH, Pantzer E, Sinka TK, DeLuca HF: J Clin Invest 64:218–225, 1979.
40. Barbour GL, Coburn JW, Slatopolsky E, Norman AW, Horst RL: N Engl J Med 305:440–443, 1981.
41. Barnes PF, Modlin RL, Bikle DD, Adams JS: J Clin Invest 83:1527–1532, 1989.
42. Honma Y, Hozumi M, Abe E, Konno K, Fukushima M, Hata S, Nishii Y, DeLuca HF, Suda T: Proc Natl Acad Sci USA 80:201–204, 1983.
43. Lemire JM, Archer DC: J Clin Invest 87:1103–1107, 1991.
44. Lemire JM, Weigle WO: J Immunol 37:3169–3174, 1986.
45. Lemire JM, Adams JS: J Bone Miner Res 7:171–177, 1992.
46. Lemire JM, Ince A, Takashima M: Autoimmunity (in press).
47. Abe J, Nakamura K, Takita Y, Nakano T, Irie H, Nishii Y: J Nutr Sci Vitaminol 36:21–31, 1990.
48. Klizumi T, Nakao Y, Matsui T, Nakagawa T, Matsuda S, Komoriya K, Kanai Y, Fujita T: Int Arch Allergy Appl Immun 77:396–404, 1985.
49. Fournier C, Gepner P, Sadouk MB, Charriere J: Clin Immunol Immunopathol 54:53–63, 1990.
50. Jordan SC, Nigata M, Mullen Y: In Norman AW, et al. (eds): Vitamin D: Molecular, Cellular and Clinical Endocrinology. New York: Walter de Gruyter, 1988, pp 334–335, 346–347.
51. Lemire JM, Archer DC, Khulkarni A, Ince A, Uskokovic MR, Stepkowski S: Transplantation (in press).
52. Yu X-P, Mocharla H, Hustmyer F, Manologas SC: J Biol Chem 266:7588–7595, 1991.