Vitamin D: its role and uses in immunology[1]

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ABSTRACT **In recent years there has been an effort to understand possible noncalcemic roles of vitamin D, including its role in the immune system and, in particular, on T cell-medicated immunity. Vitamin D receptor is found in significant concentrations in the T lymphocyte and macrophage populations. However, its highest concentration is in the immature immune cells of the thymus and the mature CD-8 T lymphocytes. The significant role of vitamin D compounds as selective immunosuppressants is illustrated by their ability to either prevent or markedly suppress animal models of autoimmune disease. Results show that 1,25-dihydroxyvitamin D_3 can either prevent or markedly suppress experimental autoimmune encephalomyelitis, rheumatoid arthritis, systemic lupus erythematosus, type I diabetes, and inflammatory bowel disease. In almost every case, the action of the vitamin D hormone requires that the animals be maintained on a normal or high calcium diet. Possible mechanisms of suppression of these autoimmune disorders by the vitamin D hormone have been presented. The vitamin D hormone stimulates transforming growth factor TGFβ-1 and interleukin 4 (IL-4) production, which in turn may suppress inflammatory T cell activity. In support of this, the vitamin D hormone is unable to suppress a murine model of the human disease multiple sclerosis in IL-4-deficient mice. The results suggest an important role for vitamin D in autoimmune disorders and provide a fertile and interesting area of research that may yield important new therapies.—DeLuca, H. F., Cantorna, M. T. Vitamin D: its role and uses in immunology. *FASEB J.* 15, 2579–2585 (2001)**

Key Words: autoimmune diseases · vitamin D as immunomodulator · inflammatory disease · vitamin D and immunity

UNTIL 1980, NO ONE had imagined that vitamin D might play a role in the functioning of the immune system. The function of vitamin D was largely considered to be in the area of calcium, phosphorus, and bone metabolism. It prevents rickets in children, osteomalacia in adults, and hypocalcemic tetany. The major thrust of research until 1980 was to determine how vitamin D functions in these important processes of mineral metabolism regulation.

In 1968, the idea appeared that vitamin D itself is biologically inactive and must be metabolically activated before it can function (1, 2). This led to the isolation and chemical identification of the active forms of vitamin D in 1968–1971 (3). Continued pursuit of the metabolism of vitamin D resulted in the understanding that vitamin D must first be hydroxylated in the liver to form 25-hydroxyvitamin D_3 (25-OH-D_3), the major circulating form of the vitamin. This form of vitamin D was subsequently found to be metabolically inactive and must be further converted to a final active form, 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) (3, 4). This last step occurs predominantly if not exclusively in the proximal convoluted tubule cells of the kidney to produce the metabolically active form of vitamin D, 1,25-$(OH)_2D_3$. This major calcium mobilizing hormone then functions directly on the enterocyte of the small intestine to markedly increase the absorption of calcium and phosphorus from the lumen into the plasma compartment. It also plays a major role in the mobilization of calcium from bone when parathyroid hormone is present. Together with parathyroid hormone, it markedly improves the renal reabsorption of calcium in the distal tubule (3, 4). These actions result in the elevation of plasma calcium and phosphorus levels to supersaturating conditions that are necessary to support mineralization of the skeleton on the one hand and prevent hypocalcemic tetany on the other (3, 4). The production of the vitamin D hormone is regulated by the need for calcium and phosphorus (4–6). Slightly low levels of plasma calcium will stimulate the parathyroids to produce and secrete the parathyroid hormone. This hormone binds to osteoblasts of bone and the entire length of the nephron of the kidney (3–5). In the kidney, parathyroid hormone stimulates the 1α-hydroxylase enzyme that produces the final vitamin D hormone. If calcium in plasma rises to very high levels, calcitonin is secreted from the c cells of the thyroid gland. This peptide hormone binds to the osteoblasts and osteoclasts to prevent the mobilization of calcium from bone; thus causing a reduction in plasma calcium levels. The exact details of this elegant endocrine system are reported elsewhere (1–5)

The attempt to understand how the active form of vitamin D carries out its functions led to the discovery of the vitamin D receptor (VDR) in 1974 and 1975 (6, 7). Before this, however, synthesis of radiolabeled 1,25-

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$(OH)_2D_3$ of high specific activity allowed for frozen section autoradiography of physiological doses of this hormone. It became clear that this hormone localized almost entirely in the nucleus in a specific fashion in target tissues. This localization was also found in other tissues not previously considered targets (8, 9). For example, keratinocytes of skin, islet cells of the pancreas, lymphocytes, and promyelocytes showed specific nuclear localization of 1,25-$(OH)_2D_3$ and the presence of the VDR. The discovery of the VDR in these tissues resulted in the idea that the vitamin D hormone had functions beyond calcium and phosphorus metabolism, which prompted investigations into the noncalcemic actions of the vitamin D hormone (10). Perhaps the most important was the discovery of the VDR in the parathyroid glands (9, 11, 12) and the demonstration that the vitamin D hormone functions through its receptor to suppress the preproparathyroid gene (13) and parathyroid cell proliferation (14). This, then, is the basis of the treatment of secondary hyperparathyroidism found in the dialysis patients and constitutes a major therapeutic application of 1,25-$(OH)_2D_3$ and its analogs.

Noting VDR in promyelocytes, Abe et al. and Tanaka et al. demonstrated that the vitamin D hormone can suppress proliferation of promyelocytes and cause their differentiation into the monocyte (15, 16). Similar effects of the vitamin D hormone on several cancerous cell lines ensued (17). A role for the vitamin D hormone in cellular differentiation thus became known. These findings prompted a search to use vitamin D analogs to treat cancer and underscored the idea that the vitamin D hormone has functions beyond calcium, phosphorus, and bone. **Table 1** provides a list of possible target cells of the vitamin D hormone including the classical sites of intestine, kidney, and bone.

The work of Manolagas and his group provided the first strong evidence that activated lymphocytes contain significant quantities of the VDR (18–20). Numerous in vitro studies followed in which the vitamin D hormone could be shown to increase proliferation or suppress proliferation, depending on the experiment and how it

TABLE 1. *1,25-(OH)2D3 target cells*[a]

Proven	Putative
1. Intestinal enterocyte	1. Islet cell, pancreas
2. Osteoblast	2. Endocrine cells, stomach
3. Distal renal cells	3. Pituitary cells
4. Parathyroid cells	4. Ovarian cells
5. Keratinocytes of skin	5. Placenta
6. Promyelocytes, monocytes	6. Epididymis
7. Lymphocytes	7. Brain (hypothalamus)
8. Colon enterocytes	8. Myoblasts (developing)
9. Shell gland	10. Aortic endothelial cells
10. Chick chorioallantoic membrane	11. Skin fibroblasts

[a] List of cells showing nuclear localization of 1,25-$(OH)_2D_3$, followed by verification of the presence of receptor by either the ligand binding assay, radioimmune sandwich assay, or ELISA assay.

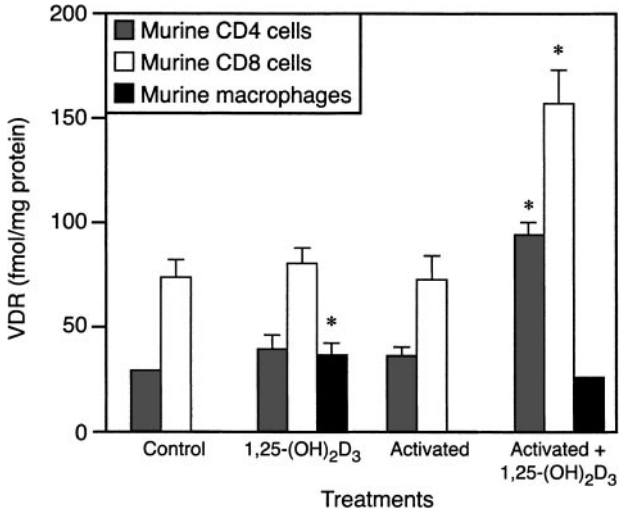

Figure 1. Vitamin D receptor (VDR) levels in the immune cells from mice. Mice were fed the Purina chow 5008 diet; lymphocytes were harvested from the spleen and separated into 95% plus pure cells as described by Veldman et al. (21).

was conducted (21). It is undeniable that peripheral lymphocytes, macrophages, and thymus tissue contain the VDR. In fact, calf thymus proved to be an excellent source of the VDR to be used for competitive binding assays in the measurement of the vitamin D hormone (22). These findings then raised the question of what effect vitamin D might have on the immune system.

In our own laboratory we determined which cells of the lymphocyte population contain the largest amounts of the VDR (21). By purifying lymphocytes to homogeneity, we were able to demonstrate that the CD-8 lymphocytes have the highest concentrations of the VDR, whether or not they were activated. Most important, the presence of 1,25-$(OH)_2D_3$ increases the amount of measurable receptor, probably because it improves the longevity and stability of the receptor in vivo as well as in vitro (23).The CD4 lymphocytes and macrophages contain relatively small but significant amounts of the VDR. **Figure 1** illustrates the VDR levels in cells of the immune system.

The next important advance in the role of vitamin D in the immune system came from studies of vitamin D-deficient mice (24). Making mice vitamin D deficient proved not to be a trivial matter, and two generations of mice reproducing under low vitamin D conditions were required. Having accomplished this, S. Yang in my group demonstrated that delayed hypersensitivity response to dinitrobenzene is impaired under conditions of vitamin D deficiency (24). This same response was also suppressed with supplemental 1,25-$(OH)_2D_3$ (25). It became evident, therefore, that T cell-mediated immunity is under modulatory control of 1,25-$(OH)_2D_3$. In its absence, T cell-mediated immune responses were blunted; with high doses of the vitamin D hormone, the same response could also be blunted. Similar immunosuppression was reported with interesting analogs of the vitamin D hormone including 22-oxa-1,25-$(OH)_2D_3$ (26) and the 16-ene-23-yne-1,25-

$(OH)_2D$ (27). These vitamin D compounds began to be known as immunosuppressants.

Based on the reported immunosuppressant activities of the vitamin D hormone, we decided to test the idea that certain autoimmune diseases involving hyperactive T cell-mediated immunity might be suitable for further study (28). We focused on the disease, experimental autoimmune encephalomyelitis (EAE) in mice. We selected B10.PL mice as the model because the sequence of neural degenerative symptoms that result in these mice after myelin basic protein injection is similar to human multiple sclerosis (MS). We succeeded in establishing EAE and showed there was an incidence of 100% in B10.PL mice fed a 0.87% calcium diet containing dietary vitamin D. However, the provision of 1,25-$(OH)_2D_3$ at 50–200 ng/day could prevent the appearance of the EAE lesions. If the vitamin D compound was given postimmunization, it prevented further development of the disease (28). By now we have carried out extensive experiments on this experimental model of autoimmune disease and have found that the vitamin D compounds (including some important potent analogs) are extremely effective in blocking the development of EAE.

In our attempt to reduce the hypercalcemic effects of 1,25-$(OH)_2D_3$, we provided this compound to animals maintained on a very low calcium diet (29). To our surprise, the low calcium diet produced a lower incidence of disease that was resistant to treatment with the vitamin D hormone (29) (see **Fig. 2**). On the other hand, when similar examinations were followed in the high calcium diet, the vitamin D compounds were effective (**Fig. 3**). Thus, it became clear that calcium is required for the vitamin D suppression of the autoimmune disease, EAE. Our initial clinical trial with the

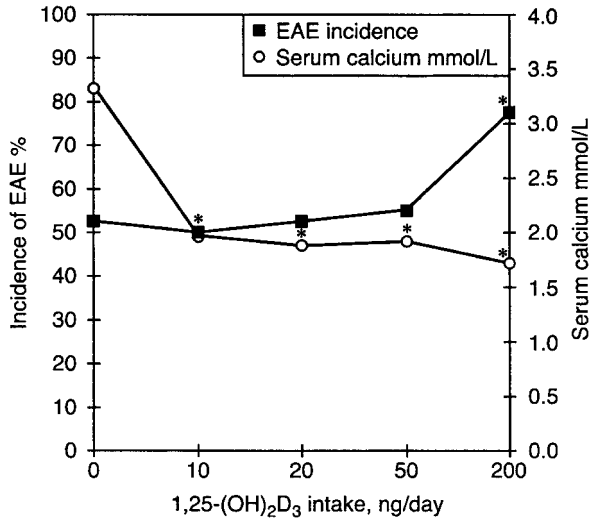

Figure 2. Female B10.PL mice were fed the 0.02% calcium, 0.3% phosphorus-purified diet and immunized with myelin basic protein together with pertussis toxin. The animals simultaneously received a daily intake of 1,25-$(OH)_2D_3$ or vehicle. Even 200 ng/day of 1,25-$(OH)_2D_3$ does not markedly reduce the incidence of EAE under low dietary calcium conditions.

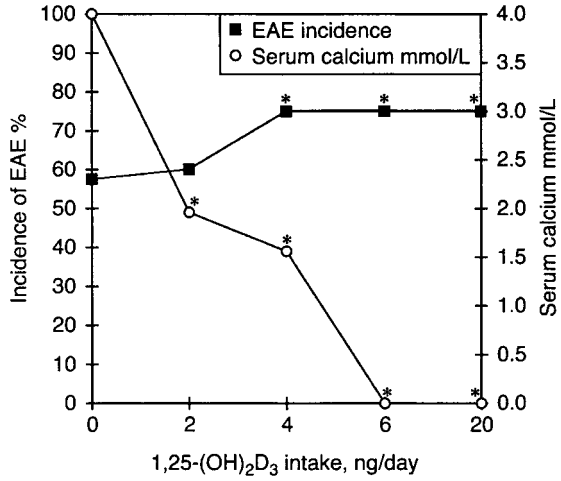

Figure 3. 1,25-$(OH)_2D_3$ is fully effective in preventing EAE in mice fed a 1% calcium, 0.3% phosphorus diet. A detailed description of the methods is found in Cantorna et al. (27).

analog 19-nor-1,25-$(OH)_2D_2$ (30) revealed little effectiveness in preventing new MS lesions primarily because we failed to simultaneously provide a normal to high calcium intake. The results, therefore, imply that a high calcium diet is required for the vitamin D hormone to be effective in the treatment of EAE.

The success with EAE suggested that vitamin D compounds could be used to treat other autoimmune disorders. Rheumatoid arthritis proved to be another example of an autoimmune disorder that can be largely prevented by the administration of the 1-hydroxylated vitamin D compounds, including 1,25-$(OH)_2D_3$ (31). Two autoimmune models of rheumatoid arthritis were used. One is the disorder caused by Lyme's disease or the organism *Borrelia burgdorferi*; the other is collagen-induced arthritis. An example of collagen-induced arthritis and its prevention by the administration of 1,25-$(OH)_2D_3$ is illustrated in **Fig. 4**. Although little more was done in studying this disorder, high calcium intakes were not required for vitamin D compounds to prevent the lesions.

Another example not yet reported in the literature from our group is systemic lupus erythematosus (SLE). In 1992, Lemire et al. described an attenuation of this disorder by 1,25-$(OH)_2D_3$ injected three times a week to MRL mice (32). However, they were unable to attenuate the rising proteinuria characteristic of this disorder and reduced the dietary calcium level in the group of mice receiving 1,25-$(OH)_2D_3$. Therefore, it is unclear whether the low calcium diet, the 1,25-$(OH)_2D_3$, or both caused the minimal improvement reported. In our research group, MRL mice were placed on either a 0.87% calcium, 0.3% phosphorus diet or a 0.02% calcium, 0.3% phosphorus-purified diet. As shown in **Fig. 5**, nearly 75% of the MRL mice developed proteinuria by 17 wk on the 0.87% calcium, 0.3% phosphorus diet; 50 ng/day of 1,25-$(OH)_2D_3$ incorporated into the diet prevented the proteinuria. As shown in Fig. 5, the severity of the MRL symptoms was also markedly prevented by the administration of

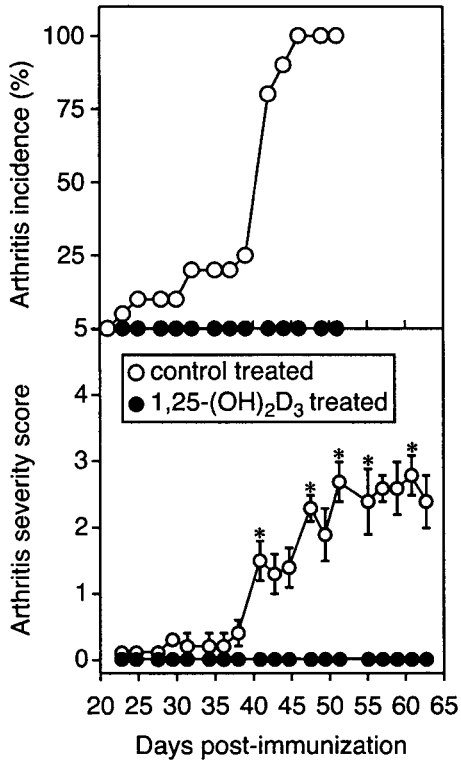

Figure 4. 1,25-$(OH)_2D_3$ prevents collagen-induced arthritis. Male DBA/1LacJ mice were fed a 0.02% calcium, 0.3% phosphorus diet. Following the immunization procedure with bovine collagen type II, animals were treated with either vehicle or vehicle containing 50 ng/day of 1,25-$(OH)_2D_3$ mixed in the diet. Mice were observed daily and began to show symptoms ~30 days postimmunization. Treated animals showed no symptoms (see Cantorna et al., ref 30).

1,25-$(OH)_2D_3$. In the 0.02% calcium diet, however, the animals receiving 1,25-$(OH)_2D_3$ developed SLE symptoms earlier and appeared to be more severely affected than animals on the 0.02% calcium diet alone (**Fig. 6**). Again, the results illustrate that a high calcium background is required for the vitamin D hormone to prevent the development of this autoimmune disorder

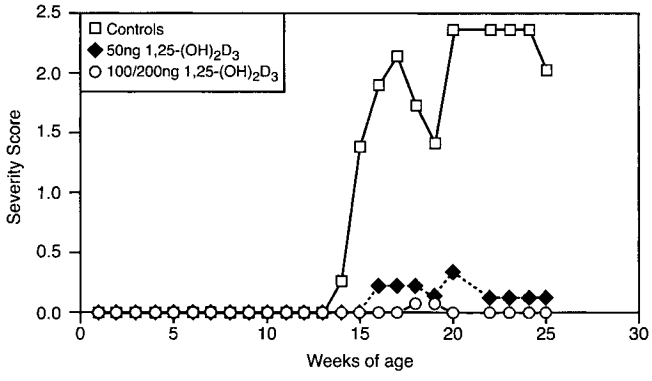

Figure 5. MRL/MJP mice were fed the 0.87% calcium, 0.3% phosphorus-purified diet throughout. Controls received vehicle incorporated into the diet or the indicated dose of 1,25-$(OH)_2D_3$ dissolved in the vehicle and added to the diet to provide 50 ng/day/mouse or 100–200 ng/day. Symptoms of lupus and proteinuria were prevented by this treatment.

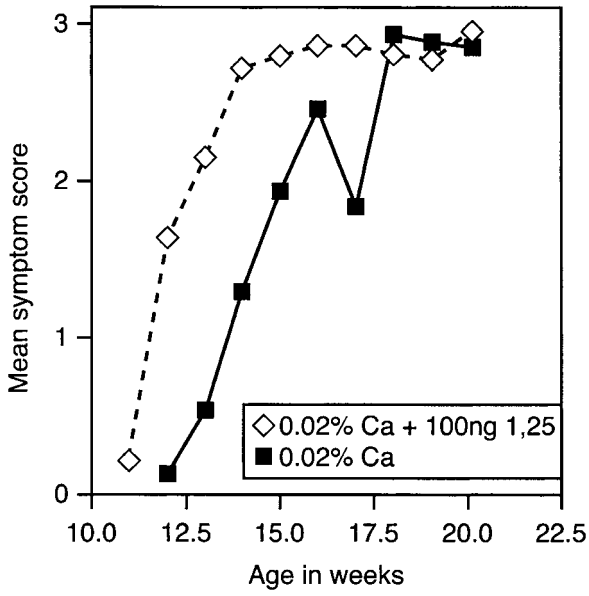

Figure 6. MRL/MJP mice fed a 0.02% calcium diet were not protected by treatment with 1,25-$(OH)_2D_3$ at 100 ng/mouse/day. This treatment in fact accelerated the appearance of lesions.

in the MRL mice. This, then, agrees with the results obtained with EAE.

The laboratory of Margherita Cantorna has investigated the possible treatment or prevention of inflammatory bowel disease (IBD) by vitamin D (33). Vitamin D deficiency accelerated the appearance of symptoms and increased the severity of IBD in interleukin 10 (IL-10) knockout (KO) mice. Vitamin D-deficient IL-10 knockout mice developed symptoms of IBD within 6–8 wk (**Fig. 7**). This was essentially prevented by the administration of 1,25-$(OH)_2D_3$, again under conditions of high calcium intakes.

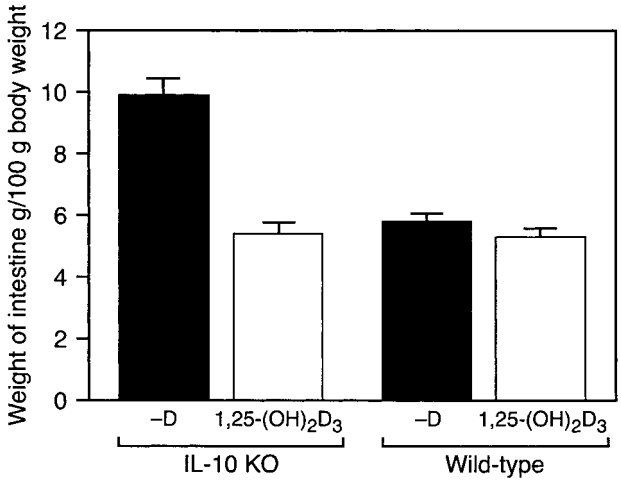

Figure 7. IL-10 knockout mice were fed the 0.87% calcium, 0.3% phosphorus diet for 10 wk and the small intestine was weighed at the end of the experiment. The data are supported by histology scores and declines in body weight. The results illustrate that 1,25-$(OH)_2D_3$ prevents the enterocolitis produced by a deficiency of IL-10.

In our own laboratory, we have investigated the development of type I diabetes in the nonobese diabetic (NOD) mouse model (34). These experiments were performed with animals on a 0.87% calcium, 0.3% phosphorus diet. Vitamin D deficiency markedly accelerated the appearance and increased the incidence of type I diabetes in the NOD mice (**Fig. 8**). Administration of ordinary vitamin D partially protected the mice from developing diabetes but the rate of incidence was still on the order of 30–40%. Addition to the diet of 1,25-$(OH)_2D_3$ at 50 ng/day for females and 200 ng/day for males prevented the appearance of the diabetic lesions. Mathieu et al. also studied the appearance of diabetes in NOD mice and have reported some partial benefit by the injection of 1,25-$(OH)_2D_3$ three times a week together with a low calcium diet (35). It is not clear whether the improvement was due to the vitamin D or due to the low calcium diet from these studies. Furthermore, the protection appeared minimal. Another major difference was that we incorporated 1,25-$(OH)_2D_3$ into the diet at all meals vs. three times a week. With a half-life of between 2 and 4 h in animals, it seems unlikely that i.p. dosing three times/wk is sufficient. In any case, the autoimmune disorder in the NOD mice can be prevented by the administration of 1,25-$(OH)_2D_3$ if animals are receiving a normal to high calcium diet.

Hypercalcemia continues to be a problem and so there is an ongoing search for a true in vivo noncalcemic analog that is still able to prevent the autoimmune disorders. So far, this has not been successful but we are only now beginning to try to prepare true noncalcemic analogs that are effective in vivo. It is possible that the calcemic action of 1,25-$(OH)_2D_3$ is also required for treatment of these autoimmune disorders.

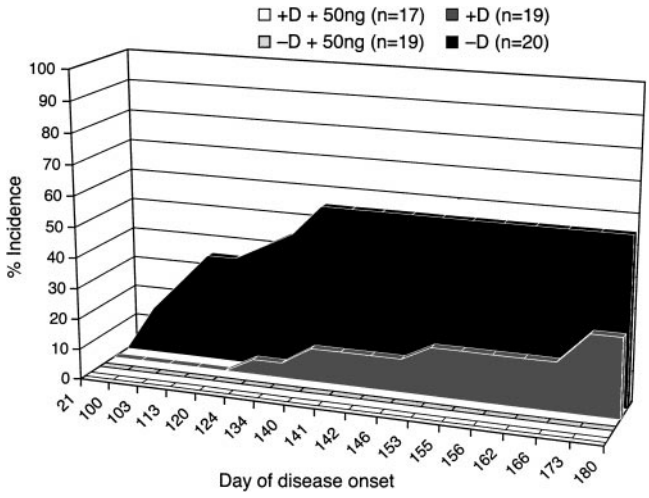

Figure 8. Incidence and severity of type I diabetes developed in NOD mice. Weanling NOD mice were fed the purified 0.87% calcium, 0.3% phosphorus diet for the entire period and, where indicated, were supplemented with either vitamin D_3 at 625 ng/day or 1,25-$(OH)_2D_3$ at 50 ng/day. Type I diabetes was diagnosed by blood glucose rising above 300 mg/100 ml. The results illustrate that vitamin D administration reduces the incidence and severity of the disease, whereas 1,25-$(OH)_2D_3$ essentially blocks diabetes in these susceptible mice (33).

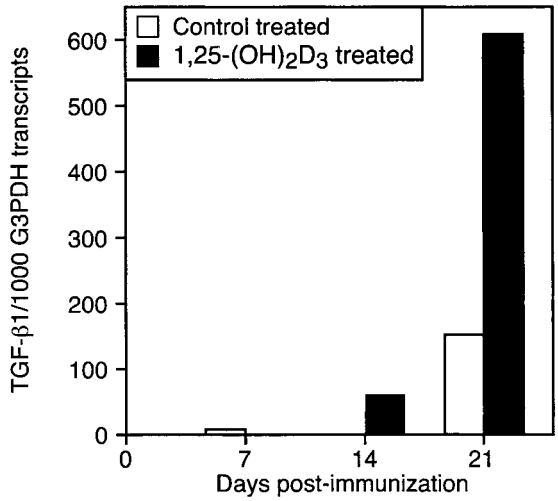

Figure 9. 1,25-$(OH)_2D_3$ treatment of B10.PL mice immunized with myelin basic protein and pertussis toxin were given either vehicle added to the diet or vehicle with 1,25-$(OH)_2D_3$ (50 ng/day for females and 200 ng/day for males). Upon the appearance of EAE symptoms in the control group, the animals were killed and lymph nodes were removed for measurement of TGFβ-1 transcripts.

Another important development has been the use of vitamin D compounds to suppress or prevent transplant rejection (36, 37). The work of Lemire et al. with a low calcium diet and 1,25-$(OH)_2D_3$ three times a week showed some improvement in maintaining transplanted organs (38). We have used the ear/heart transplant model to look at the effects of vitamin D compounds on transplant rejection. The donor mice differed from the recipient mice by two major histocompatability complex differences (allografts). After transplantation, the heart tissue in this model begins to

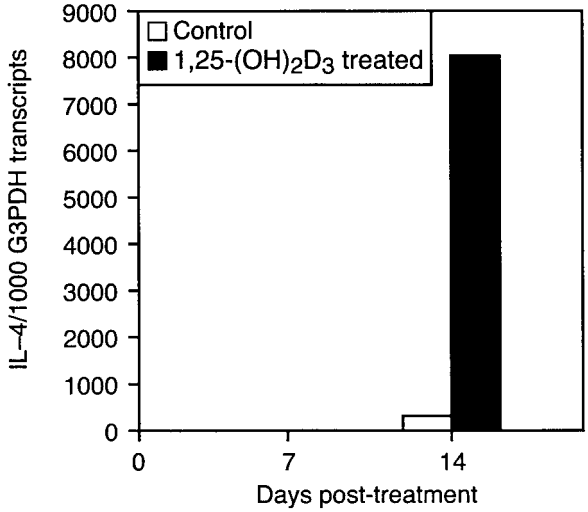

Figure 10. 1,25-$(OH)_2D_3$ supplementation markedly increases the transcripts for IL-4 in the lymph nodes of B10.PL mice immunized with myelin basic protein and pertussis toxin as described in Fig. 9 legend.

beat at 7 days. In the isografts, no rejection took place in any of the animals; within 27 days, allograft rejection was complete. Cyclosporin A at relatively low doses appeared not to protect whereas 1,25-$(OH)_2D_3$ at 50 ng/day in the diet (again, along with 0.87% calcium) protected the animal for as long as 100 days. Vitamin D compounds may be useful in preventing transplant rejection.

The sum of these findings in whole animals clearly illustrates that T cell-mediated immunity can be regulated by exogenous administration of 1-hydroxylated vitamin D compounds. The absence of vitamin D from the diet might also increase the incidence and severity of autoimmune diseases, pointing to important regulatory actions of the vitamin D hormone.

The action of 1,25-$(OH)_2D_3$ as an immunosuppressant appears to be specific since it does not appear to interfere with the ability of the animal to act defensively against opportunist infection (37). We have tested whether vitamin D treatment of mice makes them more susceptible to two infectious diseases. One was an infection caused by the fungal pathogen *Candida albicans*, a common infection found in transplant patients; the other was susceptibility to a viral herpes infection. In both cases, no interference by the vitamin D compounds was found in the disease course brought about by these organisms, whereas identical vitamin D doses were fully effective in preventing transplant rejection. It is also important to realize that vitamin D does not appear to act on the B lymphocyte, primarily because the B lymphocyte does not contain VDR in appreciable amounts (21).

There have been numerous studies of in vitro behavior of a variety of T cells and macrophages, all actors in the immune system. Although these have been contradictory and difficult to interpret, we are beginning to see some progress in understanding the mechanism whereby vitamin D acts as an immunosuppressant in vivo. We have measured the mRNAs encoding cytokines in the lymphocytes found in the lymph nodes of control mice and those treated with 1,25-$(OH)_2D_3$ (39). As illustrated in **Fig. 9** and **Fig. 10**, vitamin D administration markedly increases TGFβ-1 and IL-4 transcripts whereas there is a reduction, if anything, in interferon γ and tumor necrosis factor α gene expression. These results do not differentiate between the direct effects of vitamin D on cytokine gene expression and the indirect effects of vitamin D as regulators of other cells and genes that result in a net change in cytokine expression. With the availability of the IL-4 KO mice, we bred IL-4 KO mice onto the EAE susceptible B10.PL background. In the absence of IL-4, EAE was more severe. IL-4 KO mice with EAE are also resistant to treatment with 1,25-$(OH)_2D_3$ (40). Clearly, we are just beginning to probe the mechanisms whereby vitamin D hormone and its analogs can act as selective immunosuppressants. We do not understand the mechanism whereby the vitamin D hormone can act as an immunosuppressant especially of T cell-mediated inflammatory responses.

Despite reports to the contrary, there are no in vivo analogs that seem to act without hypercalcemia. Suppression of autoimmune disease requires not only the active form of vitamin D and its analogs, but also adequate or high calcium intakes. Further complicating the picture, little has been done to understand how vitamin D deficiency influences delayed type hypersensitivity. It is not clear whether the suppression of delayed type hypersensitivity response seen during vitamin D deficiency is due to the secondary effect of hypocalcemia or is a direct action of vitamin D on certain components of the immune system. This is a complicated and fertile area of investigation, from which may emerge important new therapies for autoimmune disease. FJ

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