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Immunomodulation by 1,25-dihydroxyvitamin D_3: therapeutic implications in hemodialysis and renal transplantation

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Abstract. The active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3, is a secosteroid hormone that regulates calcium and bone metabolism, controls cell proliferation and differentiation, and plays an important role as an immunomodulator. Recent advances in understanding the mechanisms underlying $1,25(OH)_2D_3$ immune actions expand the range of the therapeutic implications of $1,25(OH)_2D_3$ and its analogs. This review will cover the current knowledge on vitamin D-mediated immunotolerance and recent advances in vitamin D-based therapies for the treatment of autoimmune disease and the prevention of graft rejection in renal transplantation. Initiation of vitamin D-based therapies at earlier stages of chronic kidney disease may impact the immune status of patients who progress to require dialysis or transplantation. The increase in immune tolerance that appears to be moderated by vitamin D and its receptors is of particular interest. Patients may be entering these later stages of CKD with improved immune status, which can affect the approach to treatments. Further continuation of vitamin D therapy to treat secondary hyperparathyroidism may have ancillary benefits on the immune system. The possibility of multiple benefits from a single therapy is both intriguing and conceptually promising.

Introduction

In addition to its classical role in the regulation of calcium and phosphate homeostasis through its actions on bone, intestines, kidney and parathyroid gland, $1,25(OH)_2D_3$ – the active metabolite of vitamin D – also exhibits non-classical functions, including inhibiting proliferation and stimulating differentiation of both benign [Hosomi et al. 1983, Morimoto et al. 1989] and malignant cells [Abe et al. 1981], and has immunomodulatory activities [DeLuca and Cantorna 2001, Mathieu et al. 2004, Verstuyf et al. 2000]. As more patients are receiving vitamin D therapy in chronic kidney disease (CKD) Stages 3 and 4 for treatment of secondary hyperparathyroidism, it is desirable to examine the implications of continuing vitamin D therapy in dialysis or transplant patients. The increase in immune tolerance that appears to be moderated by vitamin D and its receptors is of particular interest. Patients may be entering these later stages of CKD with improved immune status, which can affect the approach to treatments. Further continuation of vitamin D therapy to treat secondary hyperparathyroidism may have ancillary benefits on the immune system. The possibility of multiple benefits from a single therapy is both intriguing and conceptually promising.

Vitamin D receptors

The biologic effects of $1,25(OH)_2D_3$, or calcitriol, are mediated by the vitamin D receptor (VDR), recognized as a member of the superfamily of nuclear hormone receptors, which act as transcription factors for specific genes [Haussler et al. 1988]. In vitamin D-responsive genes, the VDR functions as a ligand-activated transcription factor that binds to specific DNA sequence elements, thus, influencing their rate of transcription [Carlberg and Polly 1998].

Vitamin D receptors have been identified in a variety of cells with putative targets including the pancreas, stomach, pituitary, ovaries, placenta, epididymis, brain, aortic endothelium, developing myoblasts and skin fibroblasts [DeLuca and Cantorna 2001]. Vitamin D receptors have been identified in most types of immune cells [Provvedini et al. 1983], especially in antigen-presenting cells (APCs) such as macrophages [Veldman et al. 2000], and dendritic cells [Brennan et al. 1987], with

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the VDR expression and $1,25(OH)_2D_3$ receptivity in lymphocytes determined by the degree of cellular activation [Morgan et al. 2000]. Interestingly, although $1,25(OH)_2D_3$ plays a pleiotropic role as an immunomodulator, VDR-deficient mice do not exhibit major immune abnormalities, as indicated by in vitro assays of immune cell function and in vivo responses to pancreatic islet allografts [Mathieu et al. 2001]. However, mice with a deficiency in the 25-hydroxyvitamin D 1α-hydroxylase, required for $1,25(OH)_2D_3$ synthesis, showed enlarged lymph nodes in proximity to the thyroid gland and a reduced number of $CD4^+$ and $CD8^+$ T lymphocytes [Panda et al. 2001].

Vitamin D and the immune system

$1,25(OH)_2D_3$ secretion by immune cells

Several immune cells – e.g. dendritic cells [Fritsche et al. 2003] and activated macrophages [Overbergh et al. 2000a] – are able to synthesize and secrete $1,25(OH)_2D_3$. As in the kidney, the cytochrome P450 enzyme, 1α-hydroxylase, is responsible for the final hydroxylation of vitamin D to produce the active vitamin, $1,25(OH)_2D_3$ [Takeyama et al. 1997]. In the proximal tubules of the kidney, this enzyme is regulated mostly by parathyroid hormone, calcium, phosphorus, and $1,25(OH)_2D_3$ [Esteban et al. 2004]. In contrast, inflammatory stimuli, such as lipopolysaccharide (LPS) [Overbergh et al. 2000a], γ-interferon (IFN-γ) [Esteban et al. 2004, Overbergh et al. 2000a], and tumor necrosis factor-α (TNF-α) [Pryke et al. 1990] can upregulate macrophage expression of 1α-hydroxylase in vitro, suggesting a direct immune modulation of $1,25(OH)_2D_3$ synthesis. Indeed, macrophage 1α-hydroxylase is relatively unresponsive to hypercalcemia and $1,25(OH)_2D_3$ [Dusso et al. 1997]. In monocytes, IFN-γ and CD14/TLR4 binding synergistically induces expression of 1α-hydroxylase in vitro [Stoffels et al. 2006].

The enzyme for $1,25(OH)_2D_3$ catabolism, vitamin D-24-hydroxylase, also is present in activated macrophages. Some immune stimuli (e.g. IFN-γ) have been shown to interfere with 24-hydroxylase, further sustaining $1,25(OH)_2D_3$ levels at sites of inflammation [Dusso et al. 1997]. In certain inflammatory states, $1,25(OH)_2D_3$ can spill over into the systemic circulation [Dusso et al. 1994], possibly explaining the hypercalcemia that occurs in situations of macrophage overactivation, such as sarcoidosis [Barbour et al. 1981], tuberculosis [Gkonos et al. 1984], rheumatoid arthritis [Mawer et al. 1991] and lymphoma [Mudde et al. 1987]. These in vitro findings indicate that activated macrophages can produce $1,25(OH)_2D_3$, but the targets and effects of this localized hormone synthesis remain unclear [Hayes et al. 2003].

Anti-inflammatory effects of vitamin D

The role of the activated form of vitamin D, $1,25(OH)_2D_3$, as an immunomodulator has been demonstrated by its ability to prevent or suppress autoimmune diseases in animal models of systemic lupus erythematosus [Lemire et al. 1992a], encephalomyelitis [Lemire and Archer 1991], and arthritis [Cantorna et al. 1998a].

$CD4^+$ T lymphocytes comprise a key component of the immune defense and can be differentiated into 2 distinct subsets [O'Garra and Barrat 2003]. T helper 1 (Th1) cells provide protection against intracellular pathogens, while T helper 2 (Th2) cells are important in eradicating parasites, and in allergic disease processes. $1,25(OH)_2D_3$ plays an important role in shaping T cell responses by modulating cytokine transcription. $1,25(OH)_2D_3$ inhibits the production of cytokines that promote Th1 differentiation (i.e. interleukin(IL)-12 [D'Ambrosio et al. 1998]) or are products of differentiated Th1 cells (e.g. IL-2 [Alroy et al. 1995], IFN-γ [Cippitelli and Santoni 1998], or granulocyte-macrophage colony-stimulating factor (GM-CSF) [Towers and Freedman 1998]). It also stimulates cytokines that support Th2 differentiation (e.g. IL-4, transforming growth factor-β_1 (TGF-β_1)) [Cantorna et al. 1998b]. $1,25(OH)_2D_3$ has been shown to enhance the development of Th2 cells via a direct effect on naive $CD4^+$ cells [Boonstra et al. 2001]. Novel mechanisms for the anti-in-

flammatory function of $1,25(OH)_2D_3$ have been uncovered, which include its ability to regulate both the migration and the homing of Th2 cells at sites of inflammation in an animal model of asthma [Topilski et al. 2004].

Antigen-presenting cells, especially dendritic cells, appear to be key targets of $1,25(OH)_2D_3$ [Adorini et al. 2004]. Since dendritic cells play a central role in initiating immune responses, $1,25(OH)_2D_3$ also could play a powerful role in reducing immune recognition and blunting the immune response at early phases. Immature dendritic cells demonstrate tolerogenic properties (the ability to inhibit T cell responses) [Jonuleit et al. 2000]. $1,25(OH)_2D_3$ has been shown to inhibit differentiation, maturation, activation and survival of dendritic cells, leading to impaired T cell activation [Penna and Adorini 2000, van Halteren et al. 2004]. Endogenous $1,25(OH)_2D_3$ and the VDR mediate inhibition of dendritic cell maturation in vivo as part of a physiologic mechanism that reduces antigen-specific responses [Griffin et al. 2001, Dong et al. 2005, Hewison et al. 2003]. In dendritic cells, $1,25(OH)_2D_3$ up-regulates the expression of the inhibitory receptor, immunoglobulin-like transcript 3 (ILT3), which has been associated with tolerance induction, inhibition of cytokine production and the ability of dendritic cells to suppress T cell responses [Adorini et al. 2004, Chang et al. 2002, Feinberg and Silvestri 2002].

Therapeutic implications of the immunomodulatory properties of $1,25(OH)_2D_3$ and D_3 analogs

Vitamin D receptor ligands have a broad range of therapeutic applications [Nagpal et al. 2001], however, hypercalcemic effects can limit their sustained systemic administration at therapeutic doses. Different analogs of vitamin D have been developed in an effort to dissociate the antiproliferative and immunomodulatory effects of VDR ligands/activators from their effects on calcium and bone metabolism. VDR conformations provide a molecular basis for understanding the potentially selective profile of VDR agonists including vitamin D analogs [Carlberg et al. 2001].

Prevention of autoimmune disease

Type 1 diabetes

In the non-obese diabetic (NOD) mouse model, administration of $1,25(OH)_2D_3$ prevented development of type 1 diabetes (T1D) [Mathieu et al. 1994a]. NOD mice exhibit defective activation of T-suppressor cells [Serreze and Leiter 1988] and $1,25(OH)_2D_3$ administration restored defective suppressor mechanisms, as indicated by an in vitro suppressor cell assay [Mathieu et al. 1994a]. In addition, high doses of $1,25(OH)_2D_3$ significantly reduced the incidence of insulitis (the histologic lesion preceding overt diabetes) and led to a significant reduction in clinical diabetes in the treated animals compared with the control group (8 vs. 56%, $p < 0.001$). The investigators concluded that the effects on diabetes were related to the correction of the defective suppressor function, unrelated to the effects on calcium metabolism, although treatment with $1,25(OH)_2D_3$ also elevated serum calcium and osteocalcin, and decreased bone calcium content compared with the control group.

Some structural analogs of $1,25(OH)_2D_3$, such as the 14-epi analog TX527, showed reduced hypercalcemic effects and similar or enhanced immunomodulatory capacities [Mathieu and Adorini 2002, van Etten et al. 2000, 2003]. The potency of these analogs as immunomodulators was confirmed by prevention of T1D and prolongation of survival of syngeneic islet grafts in animal models without induction of hypercalcemia [Casteels et al. 1998, van Etten et al. 2003]. Slowing of diabetes progression was accompanied by an enhanced frequency in the pancreatic lymph nodes of autoantigen-specific $CD4^+$ $CD25^+$ regulatory T cells that are able to inhibit T cell responses to the pancreatic autoantigen [Gregori et al. 2002].

In addition to promoting the generation of lymphocytes with suppressive properties, $1,25(OH)_2D_3$ and its analogs could also lead to elimination of effector cells present in NOD mice by inducing a Th1 to Th2 cytokine shift (i.e. down-regulation of Th1 cytokines and up-regulation of Th2 cytokines) in the pancreas [Overbergh et al. 2000b] and/or by restoring the sensitivity of thymocytes to

apoptotic signals [Decallonne et al. 2005]. In addition, the vitamin D analog BXL-219 significantly decreases the production of inflammatory chemokines by islet cells, inhibiting the recruitment of pathogenic Th1 cells into the pancreatic islets [Giarratana et al. 2004]. The impact on clinical use has yet to be substantiated, but in animal models, vitamin D and its analogs reduce the inflammatory response, improve suppressor mechanisms and reduce the development of T1D.

Experimental autoimmune encephalomyelitis

Since most available immunosuppressants exhibit a narrow therapeutic window, a major goal in the development of immunomodulatory strategies is to discover or identify combinations of agents with synergistic effects. Synergism has been demonstrated between $1,25(OH)_2D_3$, an immunomodulator that interacts with T cells but mainly targets APCs, and typical anti-T-lymphocyte immunosuppressive agents such as cyclosporin A [Branisteanu et al. 1995, Mathieu et al. 1994b,c], rapamycin (sirolimus) [Branisteanu et al. 1997], and tacrolimus [Mathieu et al. 1994c].

In an in vitro model of T cell activation, while all combinations with $1,25(OH)_2D_3$ were synergistic in inhibiting phytohemagglutinin A-induced lymphocyte proliferation, the strongest synergy was seen with inhibitors of IL-2 secretion (i.e. cyclosporine index 0.16, tacrolimus index 0.27) [van Etten et al. 2000]. The differences in synergy seen in vitro between the immunosuppressants, between the vitamin D analogs and the immunosuppressants, and the dose-dependent nature of the synergism were confirmed in a model of experimental autoimmune encephalomyelitis [van Etten et al. 2000]. Therefore, $1,25(OH)_2D_3$ and its analogs are potent dose-reducing agents for other immunomodulators, which may have potential therapeutic applications in autoimmune disease and transplantation [Mathieu and Adorini 2002, van Etten et al. 2000].

Prevention of transplant rejection by vitamin D and its analogs – experimental models

$1,25(OH)_2D_3$ and its analogs prolong allograft survival in several experimental models, including heart [Lemire et al. 1992b], pancreatic islet [Gregori et al. 2001], small bowel allografts [Johnsson and Tufveson 1994], and bone marrow transplantation [Pakkala et al. 2001]. Inhibition of acute allograft rejection observed with $1,25(OH)_2D_3$ has been similar or even superior to that induced by treatment with optimal doses of cyclosporin A or other immunosuppressants. However, in some of the studies, the efficacy of monotherapy with vitamin D or analogs in delaying acute rejection was modest, at least in part because of dose-limiting toxic effects and the possible induction of hypercalcemia [Adorini et al. 2005]. Vitamin D analogs administered concurrently with cyclosporin A have also been shown to inhibit chronic allograft rejection, as evidenced by inhibition of inflammation in the adventitial layer and of hyperplasia in the intimal layer of rat aortic allografts [Räisänen-Sokolowski et al. 1997].

In a study of renal allotransplants in rats, $1,25(OH)_2D_3$ significantly prolonged survival following renal transplantation compared with control [Redaelli et al. 2002]. Although cyclosporin A increased survival compared with $1,25(OH)_2D_3$ alone, the combination produced the best results [Redaelli et al. 2002] (Figure 1). In addition, $1,25(OH)_2D_3$ markedly attenuated the severity of allograft rejection and led to improved allograft function. It should be noted that $1,25(OH)_2D_3$ and cyclosporin A caused differential effects on regulation of pro- and anti-inflammatory cytokines. While both $1,25(OH)_2D_3$ and cyclosporin A prevented early post-transplantation increases in IL-2 and IL-12 compared with control, only $1,25(OH)_2D_3$ led to increases in IL-10 and IL-4, providing further evidence that $1,25(OH)_2D_3$ shifts immune responses from the Th1 to the Th2 pathway, which may contribute to improved graft tolerance [Redaelli et al. 2002]. In the mouse aortic allograft model, a model of immune-mediated vascular thickening that shares similarities with human chronic allograft rejection, monotherapy with BXL-628, a non-

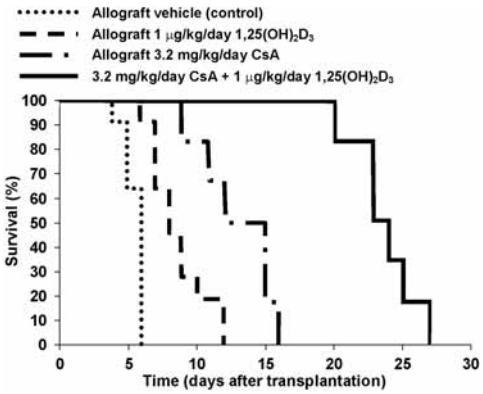

Figure 1. $1,25(OH)_2D_3$ improved survival following renal allograft transplantation in rats [Redaelli et al. 2002]. Animals were treated with intraperitoneal administrations of $1,25(OH)_2D_3$ or cyclosporin A (CsA) or a combination of $1,25(OH)_2D_3$ and CsA or control vehicle. Animals treated with $1,25(OH)_2D_3$ survived longer than animals receiving vehicle only, and the combination regimen showed an additive effect. As controls, kidney isograft transplantation and vehicle administration did not lead to recipient death. Vitamin D vs. control ($p = 0.009$); CsA vs. vitamin D ($p = 0.008$); Combination vs. CsA alone ($p < 0.001$). CsA = cyclosporin A.

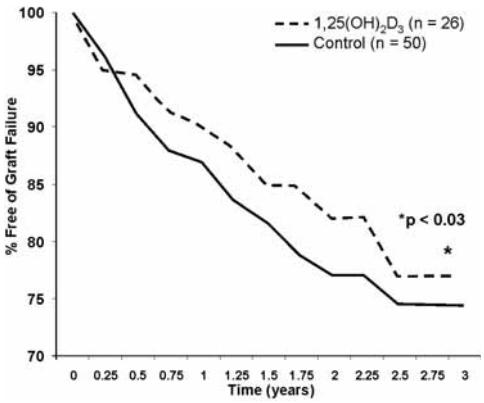

Figure 2. Graft survival in $1,25(OH)_2D_3$-treated and control patients with chronic allograft dysfunction [O'Herrin et al. 2002]. $1,25(OH)_2D_3$-treated patients with chronic allograft nephropathy (CAN) and/or increased serum creatinine levels were compared with a cohort of age- and gender-matched patients with CAN. Graft survival was prolonged in $1,25(OH)_2D_3$-treated patients compared with controls ($p < 0.03$).

hypercalcemic VDR agonist, produced significant inhibition of both acute and chronic rejection [Amuchastegui et al. 2005]. The BXL-628 effect was significantly superior to that of dexamethasone and led to a reduction of up to 80% of the aortic graft intimal hyperplasia. In addition to the possibility of a direct effect of this analog on aortic and vascular smooth muscle cells, BXL-628 monotherapy was associated with a significant reduction of leukocyte infiltration into the grafted aorta [Amuchastegui et al. 2005].

Clinical trials

Early clinical trials demonstrated beneficial immunoprotective effects of vitamin D analogs in renal transplant patients [O'Herrin et al. 2002, Tanaci et al. 2003]. However, most of the currently published clinical studies in kidney transplant recipients are retrospective analyses that do not provide definitive evidence of the putative beneficial immunological effects of vitamin D or its analogs. A small retrospective trial that evaluated the effects of $1,25(OH)_2D_3$ on allograft function in 48 patients who had undergone kidney transplantation found a significant deceleration in the rate of loss of renal graft function following treatment with $1,25(OH)_2D_3$ [Aschenbrenner et al. 2000].

In a retrospective analysis of 76 kidney and kidney-pancreas recipients receiving immunosuppressive regimens, late administration of $1,25(OH)_2D_3$ treatment (> 1 year post transplant) was associated with significantly improved graft survival and a trend toward fewer acute rejection episodes (Figure 2). Further, $1,25(OH)_2D_3$ was not associated with adverse events, and the mean serum calcium levels did not differ significantly from baseline [O'Herrin et al. 2002].

In another retrospective analysis of the effects of vitamin D on renal transplant outcomes ($n = 70$) [Tanaci et al. 2003], osteoporotic renal transplant recipients experienced fewer cases of acute rejection after $1,25(OH)_2D_3$ treatment, however, the mean length of time of graft survival did not differ between groups. Serum calcium levels increased steadily after $1,25(OH)_2D_3$ and were significantly higher than controls ($p = 0.006$). These findings suggest that, despite its potentially limiting effects on calcium and bone, vitamin D should be further investigated as an adjunct immunomodulatory therapy for kidney transplantation [Becker et al. 2002]. Ongoing studies are being conducted with the third-generation vi-

tamin D analog, paricalcitol, to evaluate its clinical utility in kidney transplant recipients.

A fourth retrospective trial in 110 renal transplant recipients, compared outcomes for 57 osteoporotic patients who received oral $1,25(OH)_2D_3$ therapy, starting a mean 22.4 months post transplantation, with 53 non-osteoporotic patients who did not [Sezer et al. 2005]. Compared with the non-treated cohort, the treated group required fewer pulse steroid doses to deal with rejection episodes and had lower creatinine and parathyroid hormone levels.

Animal studies and early clinical trials, although retrospective and not designed to show $1,25(OH)_2D_3$ effects on the immune system, strongly suggest a potential immunomodulatory role. Prospective randomized trials are necessary to identify potential benefits in treating autoimmune diseases and transplant recipients.

The therapeutic implications of vitamin D analog use in kidney transplant recipients go beyond their immunomodulatory functions. The long-term success of kidney transplantation is hampered by multiple factors, including bone disease and elevated fracture risk, increased cardiovascular morbidity and mortality, increased susceptibility to several types of cancer and chronic allograft nephropathy. Several of these factors can be potentially ameliorated by vitamin D therapy [Griffin and Kumar 2005]. The clinical benefits of $1,25(OH)_2D_3$ administration on bone mineral density and secondary hyperparathyroidism in kidney transplant recipients have been extensively documented. In addition, $1,25(OH)_2D_3$ anti-tumor effects and direct and indirect anti-fibrosis effects [Griffin and Kumar 2005], as well as the ability of vitamin D analogs to inhibit vascular intimal thickening in experimental models [Amuchastegui et al. 2005], suggest additional potential clinical benefits. There is no direct evidence that the anti-inflammatory actions of vitamin D and its analogs, or their effect on the renin-angiotensin system and the myocardium, confer any clinical protection against cardiovascular disease [Levin and Li 2005]. Nevertheless, retrospective studies have documented an increased survival rate among dialysis patients receiving vitamin D therapy compared with the control group [Levin and Li 2005, Teng et al. 2005], as well as in those patients receiving paricalcitol, a vitamin D analog, who experienced significantly less hypercalcemia compared with patients receiving $1,25(OH)_2D_3$ [Levin and Li 2005, Teng et al. 2003].

Immunomodulation by $1,25(OH)_2D_3$ in patients with renal failure

The immunomodulatory role of $1,25(OH)_2D_3$ in patients with renal failure is less clear than its role in autoimmunity and immunotolerance to transplants. It is widely accepted that uremia is an immunosuppressed state with increased susceptibility to infections and poor response to immunizations [Moe et al. 2001]. The findings of improved natural killer cell cytotoxicity, which is part of the innate immune response, and altered antigen-specific responses in patients with end-stage renal disease treated with $1,25(OH)_2D_3$ remain controversial [Moe et al. 2001]. There is still a lack of definitive evidence that either $1,25(OH)_2D_3$ or some of its analogs can enhance ex vivo immune responses in patients with end-stage renal disease [Moe et al. 2001]. However, strong data demonstrate that, when frank vitamin D deficiency is present, even poorer host defenses are observed [Cantorna et al. 2004]. Therefore, avoiding vitamin D deficiency is a priority.

Conclusions

Widespread distribution of VDRs within classical and non-classical target tissues suggest a multiplicity of physiological roles for $1,25(OH)_2D_3$. One of its most interesting roles is that of an immunomodulator which creates a potential for additional therapeutic applications for vitamin D and its analogs. Active vitamin D can exert its immunoprotective effects through several cellular mechanisms. The synergistic effects of active vitamin D analogs with currently available immunomodulators may result in better response rates for the treatment of immune disorders.

The use of vitamin D and its analogs earlier in the spectrum of CKD suggests that

patients in later stages, particularly those requiring dialysis, may have an improved immunologic profile. Since hemodialysis induces a strong inflammatory response, the use of $1,25(OH)_2D_3$ or its analogs to treat secondary hyperparathyroidism may provide an ancillary benefit in reducing inflammation.

Further, active vitamin D and its analogs offer benefits to the post-transplant therapeutic regimen through actions that allow dose reduction of other, more toxic immunomodulators as well as improved graft survival benefits. Although the mechanisms by which active vitamin D contributes to decreased acute and chronic rejection remain to be fully elucidated, promising data demonstrating a reduction in the number of rejection episodes with vitamin D treatment warrant additional studies in the future.

Finally, recent changes in the vitamin D therapy paradigm for CKD, such as the introduction of non-calcium-based phosphate binders to control hyperphosphatemia and the availability of non-hypercalcemic vitamin D analogs have broadened the therapeutic window, allowing for higher doses of vitamin D analogs without increased toxicity. However, since each vitamin D analog has a unique profile of tissue-selective effects, individual agents must be studied separately at appropriate doses to assess their effects on the immune system.

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