Effect of Active Vitamin D on Expression of Co-Stimulatory Molecules and HLA-DR in Renal Transplant Recipients

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Abstract

Objectives: **Full activation of T cells requires 2 distinct but synergistic signals. The first is the T-cell antigen receptor, which is antigen specific, and the second is activation of co-stimulatory signals. Active vitamin D (1, 25-dihydroxyvitamin D3) decreases T-cell activation and proliferation, inhibits differentiation and maturation of dendritic cells, and induces tolerogenic dendritic cells. These immunoregulatory effects may be due, at least in part, to changes in cytokine secretion and expression of co-stimulatory molecules. The use of active vitamin D has been reported to improve allograft survival, decelerate loss of allograft function, and prevent acute rejection. This study was conducted to assess the effect of active vitamin D on the expression of co-stimulatory molecules and HLA-DR in renal transplant recipients.**

Materials and Methods: **In this prospective study, we enrolled 24 renal transplant recipients who had undergone a transplant 6 to 18 months earlier, had stable allograft function, and were without episodes of allograft dysfunction or febrile illness in the previous 2 months. Participants were administered oral calcitriol 0.5 μg daily for 4 weeks. Expression of HLA-DR, CD28, CD86, and CD40 in peripheral blood leukocytes was assessed by flow cytometry before and after calcitriol administration.**

Results: **Compared to baseline levels, expression of HLA-DR decreased by 16.8%; expression of CD28, by 30%; of CD40, by 31.2%; and of CD86, by 36.7%.**

Conclusions: In renal transplant recipients, decreased expression of co-stimulatory and HLA-DR molecules occurred after treatment with active vitamin D. Such changes may be involved in increasing allograft survival.

Key words: *1, 25-dihydroxyvitamin D3; 1,25(OH)2D3; T cells; immunomodulation; allograft survival.*

Full activation of T cells requires 2 distinct but synergistic signals. The first involves the T-cell receptor, which binds to specific processed antigens in the context of the major histocompatibility complex (MHC) presented by antigen-presenting cells, and the second is activation of co-stimulatory signals, which is not antigen specific.

These surface co-stimulatory molecules cannot activate T cells on their own but rather amplify or counteract signals provided by the T cell receptor. The best known of these co-stimulatory molecules are CD28, CD80 (B7-1), CD86 (B7-2), and CD40 (CD154). In the absence of inflammation or pathogenic elements, most dendritic cells in peripheral tissues and lymphoid organs are arrested as immature phenotypes, characterized by high endocytic capacity and low surface expression of MHC and co-stimulatory molecules. However, upon interaction with an antigen or inflammatory cytokines, dendritic cells acquire an activated phenotype and up-regulate surface expression of MHC and co-stimulatory molecules. The type of immune response to an antigen strongly depends on the different activation states of dendritic cells. Presentation of an antigen by an inactive dendritic cell will lead to tolerance, as in the setting of neoplastic antigens. In contrast, presentation of antigen by an active dendritic cell will cause an inflammatory response (1). Interventions in the co-stimulatory pathways for tolerance induction are

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among the newest strategies in organ transplant and treatment of autoimmune disorders (2-5).

In addition to its role in calcium and bone metabolism, active vitamin D has important immunoregulatory effects. Vitamin D receptors have been found in many cells of the immune system, especially macrophage and dendritic cells as well as CD4+ and CD8+ lymphocytes (6, 7). In vitro, active vitamin D decreases T-cell activation, blocks mitogen-stimulated T-cell proliferation (8) of cytotoxic T lymphocytes, inhibits differentiation and maturation of dendritic cells, and induces tolerogenic dendritic cells (9, 10). These immunoregulatory effects may be due at least in part to changes in cytokine secretion and expression of co-stimulatory molecules. Active vitamin D down- regulates expression of co-stimulatory molecules in monocytes and dendritic cells in culture media (11,12).

It has been proposed that reduced expression of co-stimulatory molecules, together with increased interleukin 10 (IL-10) and decreased IL-12 secreted by dendritic cells after exposure to active vitamin D, may increase regulatory CD4+CD25+ T cells (13). Moreover, exposure of myeloid dendritic cells to active vitamin D up-regulates production of Chemokine (C-C motif) ligand 17 (CCL17) that attracts regulatory T cells (10). These T cells may mediate tolerance induction by their contact-dependent or autocrine and paracrine inhibitory roles in proliferation of activated T cells and blockade of production of IL-2 and interferon-gamma by the activated T-cell population (14,15).

There are reports of improved allograft survival, deceleration in the loss of allograft function, and prevention of acute rejection with the use of calcitriol and recent less calcemic derivatives in animals and human organ transplant (16-23). This study was conducted to assess the effect of active vitamin D on the expression of co-stimulatory molecules in renal transplant recipients.

Materials and Methods

In this prospective study, we enrolled 24 renal transplant recipients who were regularly followed at our transplant clinic, had undergone a transplant 6 to 18 months before the study, had stable allograft function (glomerular filtration rate > 60 mL/min by the Cockroft and Gault formula), and were without episodes of acute allograft dysfunction or febrile illness in the previous 2 months. The participants were administered calcitriol 0.5 μg orally per day for 4 weeks. All patients had received cyclosporine, mycophenolate mofetil, and prednisolone. The dosage of immunosuppressive drugs was not changed during the study. Patients were excluded if they had induction with anti-IL-2 receptor blockers or antilymphocyte globulin or if they had a history of vitamin D administration.

Expression of HLA-DR, CD28, CD86, and CD40 on peripheral blood leukocytes was assessed by flow cytometry (Partec Particle Analyzing System, Münster, Germany) in samples obtained before and after 4 weeks of calcitriol. Staining procedures were performed using fluorescein isothiocyanate-conjugated monoclonal antibodies for HLA-DR (Clone AB3), CD28 (Clone CD28.1), CD86 (Clone BU 63), and CD40 (Clone LOB 7/6) (Dako, Glostrup, Denmark). Samples were processed within 3 hours of collection. Peripheral venous blood was collected into test tubes containing anticoagulants. In different test tubes, 100 μL of blood was mixed with 10 μL anti-human CD-fluorescein isothiocyanate. Test samples were incubated in the dark at 4°C for 30 minutes, then incubated for 10 minutes after adding 1000 μL lysis reagent A (Dako), and finally for 10 minutes after adding 100 μL lysis reagent B (Dako). Samples were washed twice with phosphate-buffered saline containing 2% bovine serum albumin. The cells were resuspended in an appropriate volume of phosphate-buffered saline and fixed by 0.3 mL 1% paraformaldehyde in 0.01 mL/L phosphate-buffered saline at pH 7.4. Test samples were analyzed by flow cytometry, and the percentage of leukocytes with the expressed marker was counted in at least 15 000 to 30 000 leukocytes.

The study was approved by the university ethics committee and written informed consent was provided by all participants. The patients were seen every 2 weeks during the study to detect adverse effects, allograft dysfunction, or febrile episodes. Serum calcium was checked in all patients 2 weeks after beginning calcitriol and at the end of the study.

Continuous data are presented as means ± standard deviation (SD). Statistical comparisons were performed using the *t* test and the Wilcoxon rank test. Data were considered statistically significant when the value for *P* was < .05. Analyses were performed with SPSS (Statistical Product and Services Solutions, version 11.0, SPSS Inc, Chicago, IL, USA).

Results

A total of 24 patients were enrolled. Mean age was 37.8 ± 11.9 years, and 18 of 24 patients (75.0%) were men. Mean transplant duration was 11 months. Data about the dosage of immunosuppressive drugs is shown in Table 1. All 24 patients finished the 4-week study.

Table 1. Profile of immunosuppressive drugs.

Mean of prednisolone	9.5 (mg/d)
Mean of cyclosporine	3.2 (mg/kg/d)
Mean of cyclosporine whole blood tough level	225 (ng/mL)
Mean of mycofenolate mofetil	1714 (mg/d)

Diltiazem was routinely administered in most of patients.

Expression of HLA-DR and other co-stimulatory molecules decreased significantly (Table 2). Expression of HLA-DR was reduced by 16.8%. Expression of CD28 was reduced by 30.0 %; of CD40, by 31.2%; and of CD86 (B7-2), by 36.7%. For example, 2.30% ± 0.74% of peripheral blood leukocytes expressed CD28 molecules at baseline, and this was reduced to 1.61% ± 0.91% after calcitriol administration (P = .004).

Table 2. Changes of co-stimulatory and HLA-DR molecules expression before and after calcitriol adminstration.

Variable	Before calcitriol Mean ± SD*	After calcitriol Mean ± SD	Percentage reduction	*P* value
CD 28	2.30±0.74	1.61±0.91	30%	.004
CD40	3.07±1.51	2.11±1.71	31.2%	.0001
CD86	2.37±1.00	1.5±0.78	36.7%	.0001
HLA DR	9.99±3.02	8.31±2.93	16.8%	.0001

*Percentage of peripheral blood leukocyte with expressed marker (counted in at least 15 000 peripheral blood leukocytes by flow cytometer)

There were no febrile episodes or acute allograft dysfunction during the study. Hypercalcemia was not seen during the study. Mean serum calcium was 2.34 mmol/L before and 2.37 mmol/L after calcitriol administration. Mean serum creatinine was 99.4 μmol/L before and 103.8 μmol/L after intervention. The differences were not statistically significant.

Discussion

The most important finding of this study was the substantial decrease in white blood cells expressing HLA-DR and co-stimulatory molecules after active vitamin D treatment. Because the proportional change multiplies to the huge number of peripheral circulating leukocytes, this represents a significant change in the absolute number of circulating white blood cells with a given expressed marker. Moreover, we did not separate any particular type of white blood cell during flow cytometry, and we assume that these changes would have been more pronounced if the changes were assessed specifically in circulating monocytes, dendritic cells, and lymphocytes.

We studied the effect of active vitamin D in patients who had undergone a transplant 6 to 18 months before the study to ensure more stable allograft function; in fact, no changes in the immunosuppressive drug regimens were needed during the study. Based on the time after transplant, the effect of active vitamin D may be different. This point may require further study.

In a study by Clavreul and colleagues (11), monocytes were cultured for 3 days with and without active vitamin D and analyzed by flow cytometry for surface expression of CD80, CD86, and class II MHC antigens. While expression of CD80 molecules was unchanged, expression of CD86 was reduced by 52%, and expression of class II MHC antigens was reduced by 59%. In a study by Tokuda and colleagues of cultured human peripheral blood monocytes, HLA-DR expression was reduced significantly that is the percentage of positive cells decreased from 90% to 55% (24). The inhibitory effect was dose-dependent and directed toward HLA class 2 antigens but not class 1 antigens.

Another point that may have an important influence on the effect of active vitamin D is the age of patients. Aging is associated with down-regulation of CD28 expression. Decreased CD28 (it can be changed to CD28 neg) cells are the biologic indicator of the aging immune system in humans and may be a reason for lower functional activity of T cells in elderly patients (25). Therefore, the impact of active vitamin D on the expression of co-stimulatory molecules and probably on reducing the risk of rejection and promoting allograft survival may be different in different age groups. The number of patients in our study was not sufficient to assess the effect of active vitamin D according to age, and further studies are needed.

CD4+CD28- T cells differ from CD4+CD28+ cells in several ways. In CD4+CD28- T cells, expression of IL-2 receptors after activation is short lived, and they do not express the CD40 ligand (26). Expression of CD40 ligand and its binding to CD40 up-regulates

expression of CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells and makes a positive stimulatory loop for T-cell activation. It is not clear whether active vitamin D directly down-regulates expression of CD86 or whether this effect is secondary to reduced expression of CD28 and/or CD40 molecules. Ligation of CD40 to its ligand provides one of the strongest activation signals for dendritic cells that cause increased production of IL-12 and shift of the T-cell response to helper 1 T cells (TH1).

Reduced expression of CD40 in dendritic cells may have a role in decreased production of IL-12 by myeloid dendritic cells that occurs after exposure to active vitamin D (27). Moreover, binding of CD40 to its ligand in B cells is crucial for immunoglobulin class switching and B-cell stimulation. Expression of CD40 was substantially reduced after exposure to active vitamin D in our study. This indicates that active vitamin D not only reduces expression of CD40 ligand in T cells, probably by induction of CD4+CD28 null T cells, but that it also may decrease expression of CD40 in dendritic cells.

In this study, we have shown decreased expression of co-stimulatory and HLA-DR molecules in renal transplant recipients treated with active vitamin D. Together with other influences of vitamin D on the immune system, this decreased expression may play a role in increasing allograft survival, as reported by other studies (16-22). Hypercalcemia as a known adverse effect of active vitamin D administration was not detected in our study population. Studies to determine the safest and most effective dose of active vitamin D for this purpose are needed. Moreover, in recent years, with the advent of less calcemic vitamin D derivatives and particularly compounds with enhanced potency of vitamin D effects on the immune system, the place of vitamin D derivatives in transplant and tolerance induction may change in the future (28-30).

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