Published in final edited form as:

Transplantation. 2015 July ; 99(7): 1365–1371. doi:10.1097/TP.0000000000000677.

Effect of absent immune cell expression of Vitamin D receptor on cardiac allograft survival in mice

Samuel J. Gavzy¹ and Peter S. Heeger²

Translational Transplant Research Center, Division of Nephrology, Dept. of Medicine, The Immunology Institute, and the Recanati-Miller Transplant Institute, Icahn School of Medicine at Mount Sinai, New York, NY, 10029

Abstract

INTRODUCTION—Vitamin D (VD) has immunomodulatory properties, but whether immune cell expression of the vitamin D receptor (VDR) impacts costimulatory blockade induced cardiac allograft survival is not known.

METHODS—To localize effects of VDR deficiency to hematopoietic cells and to avoid the metabolic consequences of systemic VDR-deficiency, we produced bone marrow (BM)-chimeric mice by transplanting lethally-irradiated C57BL/6 mice with congenic VDR^{-/-} or WT bone marrow. Following reconstitution, we characterized baseline immune profiles and transplanted chimeras with heterotopic cardiac allografts with or without costimulatory blockade using anti-CD154 (MR1) or CTLA4Ig, the latter approved for use in human kidney transplant recipients.

RESULTS—Immune reconstitution occurred equivalently in chimeras with WT and VDR^{-/-} BM. Untreated animals rejected class-II-disparate and fully allogeneic cardiac transplants with similar kinetics. Compared to untreated controls, treatment with either MR1 or CTLA4Ig induced significant and equivalent prolongation of graft survival in both groups of chimeric recipients. We observed no differences in induced anti-donor cellular or humoral alloimmunity between groups.

CONCLUSIONS—Our findings support the conclusion that absent immune cell VDR expression a) does not impact the strength, phenotype or kinetics of heart transplant rejection in mice and b) does not impact the graft-prolonging effects of costimulatory blockade including that induced by clinically employed CTLA4Ig.

Introduction

Vitamin D (VD), whether ingested or synthesized in the skin from 7-dehydrocholesterol following UV exposure, undergoes 25-hydroxylation in the liver to yield 25-hydroxyvitamin D [25(OH)D], the principal circulating form of the vitamin (reviewed in (1, 2)). Subsequent 1-hydroxylation predominantly occurs in the kidney via the action of CYP27B1, resulting in the formation of active 1,25-dihydroxyvitamin D [1, 25(OH)₂D]. 1,25(OH)₂D functions by

Corresponding Author: Peter S. Heeger, Mount Sinai School of Medicine, One Gustave Levy Place, Box 1243, NY, NY 10029, Telephone: 212-241-6324, Fax: 212-987-0389, peter.heeger@mssm.edu.

¹S Gavzy performed and analyzed experiments, contributed to writing and editing. sgavzy@gmail.com

²P Heeger oversaw and directed all aspects of the work, analyzed data, wrote and edited manuscript. peter.heeger@mssm.edu

Disclosure: The authors declare no conflicts

binding to an intracellular vitamin D receptor (VDR) within target tissues, and after translocation to the nucleus hetero-dimerizes with the retinoid X receptor (RXR). The $1,25(\text{OH})_2\text{D}/\text{VDR}/\text{RXR}$ complex interacts with VDR response elements to mediate transcription of various response genes.

While concepts regarding the function of vitamin D (VD) have traditionally focused on calcium homeostasis and bone metabolism, a growing body of evidence indicates that VD has potent immune-modulatory properties. T-cells, macrophages and dendritic cells (DCs), among other immune cells, express the VDR and the 1-hydroxylase CYP27B1, the latter of which permits localized synthesis of $1,25(\text{OH})_2\text{D}$ (1–5). Such intracellular production of $1,25(\text{OH})_2\text{D}$ has been shown to modulate the function of murine and human macrophages, T-cells and dendritic cells (DCs) through a variety of mechanisms. In monocytes and macrophages, locally produced $1,25(\text{OH})_2\text{D}$ upregulates antibacterial proteins, including cathelicidin and β -defensin 2, molecules that have been linked to control of tuberculosis infections (1, 2, 6). In contrast to this well-documented, VD-induced, enhancement of innate immunity, evidence indicates that intracellular synthesis and action of $1,25(\text{OH})_2\text{D}$ restrains adaptive immune responses. Therapeutic administration of $1,25(\text{OH})_2\text{D}$ abrogated phenotypic expression of murine models of diabetes, colitis, and experimental allergic encephalomyelitis, among others (7–14). Results of in vitro analyses revealed that these effects are in part caused by the ability of $1,25(\text{OH})_2\text{D}$ to inhibit T cell proliferation, skew T cell cytokine profiles from Th1 toward Th2, limit Th17 production, inhibit DC maturation, limit responsiveness of DCs to proinflammatory stimuli, and facilitate induction of regulatory T cells (Treg) (2, 5). Consistent with an immunosuppressive effect, the adoptive transfer of allogeneic DCs pretreated ex vivo with $1,25(\text{OH})_2\text{D}$ prior to murine skin transplantation did not accelerate, and in fact prolonged, allograft survival (15, 16).

While these findings support the concept that therapy with VD can be immunosuppressive, the physiological role of intact VDR signaling in immune cells in vivo remains less clearly defined. Immunological analysis of mice with genetically induced VDR deficiency ($\text{VDR}^{-/-}$ mice) show inconsistent results dependent upon the model studied, potentially because the metabolic abnormalities associated with VD deficiency, including hypocalcemia, alter immune function (2).

Unmanipulated $\text{VDR}^{-/-}$ mice develop worsened autoimmune colitis yet reject MHC-disparate islet allografts with similar kinetics to their WT counterparts (17, 18). These published findings were not designed to specifically test the effects of absent VDR in immune cells on allograft rejection, and did not address the effects of absent VDR on fully vascularized cardiac allograft rejection. Furthermore, whether VDR deficiency negatively impacts costimulatory blockade-induced prolongation of allograft survival has not been reported.

To address these issues we performed a series of experiments using bone marrow (BM) chimeric animals, in which we reconstituted lethally irradiated WT mice with BM from $\text{VDR}^{-/-}$ donors (or WT controls) such that VDR deficiency was confined to the BM compartment. This strategy bypasses the adverse effects of VDR-deficiency on bone formation and animal health. Following immune reconstitution and verification of baseline

immune phenotypes, we transplanted the chimeras with heterotopic cardiac allografts in the presence or absence of costimulatory blockade to assess the impact of immune cell VDR-deficiency on alloimmunity and graft survival in vivo.

Materials and Methods

Animals

B6.129S4-Vdr^{tm1Mbd}/J (VDR^{-/-}, CD45.2, H-2^b) and B6.C-H2-Ab1^{bm12}/KhEg-Mc1r^{e-J}/J (bm12, CD45.2, H-2^{bm12}) mice were bred from animals purchased from The Jackson Laboratories (Bar Harbor, ME). C57BL/6 (B6, CD45.2, H-2^b), B6.SJL-Ptprca Pepcb/BoyJ (CD45.1, H-2^b), and BALB/c (CD45.2, H-2^d) mice were purchased from The Jackson Laboratories at 6-10 weeks of age. All mice were housed in the Icahn School of Medicine at Mount Sinai Center for Comparative Medicine and Surgery in accordance with guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International.

VDR^{-/-} mice are not viable breeders. Offspring of VDR^{+/-} breeder pairs were genotyped using Sigma EXTRACT N Amp Kit (Sigma Life Science, St. Louis, MO) with the following primers: WT Forward (oIMR5219): 5' CTC CAT CCC CAT GTC TCT TT 3'; Common (oIMR5220): 5' TTC TTC AGT GGC CAG CTC TT 3'; and Mutant (oIMR2060): 5' CAC GAG ACT AGT GAG ACG TG 3'. Amplified DNA samples were run alongside known controls for WT, +/-, and -/- on 2% E-gel SyberSafe (Invitrogen, Grand Island, NY).

Bone marrow (BM) chimeras

BM cells were collected from the long bones of WT or VDR^{-/-} B6 (CD45.2) mice. Recipient WT B6 (CD45.1) mice were irradiated with 2 doses of 6 Gy, (600 rad, Mark I Model 137Cs irradiator, JL Shepherd & Associates, San Fernando, CA) 3 hours apart. Four hours following the final dose, recipient mice received 2×10⁶ BM cells i.v. Chimerism was tested 8 weeks later by comparing CD45.1 and CD45.2 expression on peripheral blood cells by flow cytometry.

Serum 25-OH-Vitamin D Measurements

Serum samples obtained from transplanted chimeras and WT controls were tested for 25(OH)D (Nationwide Laboratory Services, Ft Lauderdale, FL, Diasorin Vitamin D assay).

Surgical Procedures

Heterotopic heart transplantation was performed as described (19, 20) by the microsurgery core facility at the Icahn School of Medicine at Mount Sinai. In some experiments recipient mice received anti-CD40L mAb (200µg - 500µg as indicated, Bio X Cell, West Lebanon, NH) i.v. with 10⁷ donor (BALB/c) spleen cells on the day prior to transplant or CTLA4Ig (500µg Bio X Cell) on day 2 following transplantation.

Graft heartbeat was monitored daily by palpation, and rejection was defined as the day on which a palpable heartbeat was no longer detectable. Rejection was verified by examination of H&E stained graft tissue sections. H&E staining was performed by the histology core facility at the Icahn School of Medicine at Mount Sinai.

Antibodies and Reagents

All antibodies for flow cytometry were purchased from BD Pharmingen (San Diego, CA) or eBioscience (San Diego, CA). Carboxy-fluorescein diacetate succinimide ester (CFSE) was obtained from Molecular Probes, Invitrogen (Carlsbad, CA).

Flow Cytometry

Samples were collected using a FACSCanto II (BD Biosciences) flow cytometer and analyzed with FlowJo software (TreeStar Inc., Ashland, OR). To measure donor specific T cell reactivity, single cell suspensions of spleen cells obtained from transplanted animals (or controls) were lysed of RBCs (10% 1.5M NH₄Cl, 0.2% 0.5M KHCO₃, and 0.02% 0.5M EDTA) and incubated in RPMI Complete Media (Invitrogen, Grand Island, NY), 10% Fetal Bovine Serum (Thermo Scientific, Waltham, MA), 1% Penicillin/Streptomycin (Lonza, Allendale, NJ), 1% L-Glutamine (Invitrogen) at 37°C, 5% CO₂, with CFSE-labeled donor-type or syngeneic spleen cells (CFSE was used to identify stimulators and gate them out of the analysis). After 17 hours, the cultures were treated with Brefeldin-A (BD BioSciences) for 2.5 hours, washed and stained with anti-CD45.1, anti-CD45.2, anti-CD4, and anti-CD8 antibodies. Intracellular IFN γ was measured by standard techniques (permeabilizing buffers from BD Biosciences and antibodies from BD Biosciences and eBioSciences). Data were collected by flow cytometry gating on the CFSE^{neg} CD4⁺ and CD8⁺ responder cells.

Alloantibody Detection

Serially diluted serum samples were incubated with donor thymocytes as targets. After a wash step the bound antibody was detected with fluorescein isothiocyanate (FITC)–conjugated rat anti-mouse IgG (eBioscience, San Diego, CA) and quantified by flow cytometry.

Immunization

BM Chimeras were immunized with 0.2mL of OVA protein (0.5mg/mL) dissolved in complete Freund's Adjuvant (CFA).

ELISPOT assays

IFN γ ELISPOT assays were performed as described (19, 20). Briefly, single cell suspensions of spleen cells were cultured for 24 hours in HL-1 media (1% Penstrep 1% L-Glutamine) alone, with one of three titrations of OVA protein (10, 3, and 1 μ g/mL), cardiac myosin protein (specificity control), or Concanavalin A (positive control) in MultiScreen_{HTS}-IP ELISPOT plates (Millipore, Billerica, MA) precoated with anti-IFN γ coating antibody (BD Pharmingen). The plates were washed, and secondary rat anti-mouse IFN γ antibody (BD Pharmingen) was added, followed by an alkaline phosphatase-conjugated anti-biotin antibody (Vector Laboratories, Burlingame, CA, USA) diluted 1:2000 in PBS supplemented with 0.1% Tween and 1% bovine serum albumin (BSA) was added for 90 min, the plates were developed by addition of 1-Step NBT/BCIP substrate (Thermo Scientific, Rockland, IL, USA), and the resulting spots were counted on an ImmunoSpot Series 3 Analyzer (Cellular Technology Ltd., Shaker Heights, OH, USA).

Statistical Analysis

Graft survival was analyzed using Kaplan Meier methods and compared using the log-rank test. Where appropriate for small sample sizes (n of 3–4 mice/group), continuous variables were compared using nonparametric methods (Mann Whitney test when comparing 2 groups and Kruskal-Wallis test when comparing 3 groups). $P < 0.05$ was considered statistically significant. All statistical evaluations were performed using Prism (GraphPad Software, La Jolla, CA).

Results and Discussion

To assess the impact of immune cell VDR-deficiency on alloimmunity and transplant survival, we produced BM chimeras by transplanting BM cells from CD45.2⁺ VDR^{-/-} or control CD45.2⁺ WT donors into lethally irradiated, congenic CD45.1⁺ WT hosts. Eight weeks later, we observed that >90% of peripheral blood cells in both groups were donor-derived (Fig 1A-B). This finding confirmed the efficacy of chimera generation and indicated that the absence of VDR in BM cells does not alter BM cell reconstitution. Importantly, all mice were fed identical VD-enriched chow and serum 25(OH)D levels were similar and within the normal range in all animals tested from both groups of chimeras (Fig 1C).

We sacrificed animals from both groups and phenotyped the spleen cells by flow cytometry (Fig 2). The analysis revealed similar frequencies of total, naïve (CD62L^{hi} CD44^{lo}), and memory (CD62L^{lo} CD44^{hi}) CD4⁺ and CD8⁺ T cells (Fig 2A-B), as well as B220⁺ B cells (Fig 2C). Similarly, when we phenotyped splenic myeloid cell subsets of the WT→WT and VDR^{-/-}→WT chimeras using CD11c and CD11b expression, and examined class II MHC and costimulatory molecule CD86 expression patterns on the subsets (as well as on B cells), we observed no significant differences between groups (Fig 3A-C).

To test whether immune cell expression of VDR impacts CD4⁺ T cell-dependent transplant rejection, we transplanted WT→WT and VDR^{-/-}→WT chimeras with MHC class II-disparate bm12 heart grafts (Fig 4A). These experiments revealed that both sets of chimeras rejected bm12 hearts with equivalent kinetics, with median survival times (MSTs) of 35–36 days ($p=0.28$). Histological examination of graft tissue showed typical mononuclear cell infiltration characteristic of rejection in both groups (data not shown).

We next tested whether immune cell expressed VDR is required for costimulatory blockade induced prolongation of cardiac allograft survival (Fig 4B). We transplanted fully MHC-disparate hearts into groups of WT→WT and VDR^{-/-}→WT chimeras with or without donor specific transfusion (DST) plus anti-CD154 mAb, MR1. DST+MR1 at either of 2 doses (200 or 500 µg) equally prolonged transplant survival between the two sets of chimeras as compared to untreated controls (Fig 4B). Similarly, we observed equivalent prolongation of graft survival in WT→WT and VDR^{-/-}→WT chimeric recipients following a single dose of CTLA4Ig (Fig 4C).

To specifically assess whether the absence of VDR in immune cells impacted the strength of the induced alloresponses in animals treated with DST+MR1, we sacrificed untreated transplant recipients and transplant recipients treated with CTLA4Ig, at the time of rejection,

and performed in vitro immune assays (Fig 5). We measured donor-reactive, IFN γ -producing CD4⁺ and CD8⁺ T cells in recipient animals by stimulating recipient spleen cells with donor (BALB/c) or control (B6) splenic antigen presenting cells (APCs) overnight, staining for intracellular IFN γ and quantifying the frequency of IFN γ -producing CD4⁺ and CD8⁺ T cells by flow cytometry. These assays revealed that while costimulatory blockade reduced the frequency of donor-reactive, IFN γ -producing CD8⁺ T cells compared with untreated recipients, we did not observe significant differences between the WT and VDR^{-/-} chimeric groups (Fig 5A-C). Further analysis (Fig 5D) showed that ~85% of the donor-reactive IFN γ -producing CD8⁺ T cells derived from the donor BM cells (i.e. CD45.2⁺ VDR^{-/-} CD8 cells in the CD45.2⁺VDR^{-/-} → CD45.1⁺WT chimeras). Measurements of anti-donor IgG in the sera of CTLA4Ig-treated recipients revealed no significant differences between groups (Fig 5E).

Finally, to test whether BM cell expression of VDR impacts T cell responses to a different (non-transplant) stimulus, we immunized groups of WT → WT and VDR^{-/-} → WT chimeras with ovalbumin (OVA) mixed in CFA and quantified the number of OVA-reactive IFN γ -producers in the spleens 2 weeks later by ELISPOT (Fig 5F). These assays showed strong but equivalent frequencies of OVA-reactive spleen cells in the 2 groups. No responses were detected in unimmunized chimeras (data not shown).

Our findings demonstrate for the first time that BM cell expression of VDR is not required for costimulatory blockade-induced prolongation of heterotopic cardiac transplant survival. Using BM chimeras we showed that animals with VDR^{-/-} BM cells but normal serum 25(OH)D levels (Fig 1), rejected MHC-disparate heart allografts with similar kinetics to WT chimeric controls, in the presence or absence of costimulatory blockade targeting CD40/CD154 (MR1) or CD28/CD80/CD86 (CTLA4Ig) interactions (Fig 4). We also observed that the strength of donor-alloreactive T cell and the induced alloantibody responses (Fig 5) were similar between groups. Furthermore, BM-cell VDR expression did not affect the strength of the T cell responses induced to the model antigen OVA (Fig 5E), extending work by others performed in VDR^{-/-} recipients (12, 17, 18).

In contrast to our negative findings, others have provided evidence that immune cell VDR expression can regulate T cell immunity in disease models that differ significantly from allograft rejection (21, 22). One such study showed that regulatory, intra-epithelial CD8⁺ T cells of the intestine function aberrantly in the absence of cell-intrinsic VDR expression, resulting in murine colitis (21). This finding among other published data (8, 9, 12, 17, 21) suggest that constant exposure of the gut to commensal microorganisms requires specific regulatory mechanisms, including VDR signaling, to prevent pathogenic, mucosal, inflammatory responses. Such regulatory mechanisms are unlikely to be engaged in the sterile heart transplant environment, and to our knowledge there is no evidence that CD8⁺ T cells contribute to costimulatory blockade-induced heart transplant survival. Together, these concepts likely account for the differences in the observed effects of BM cell VDR deficiency between the experimental systems. Another research group showed that pharmacologic doses of vitamin D inhibit murine experimental autoimmune encephalomyelitis through a mechanism dependent upon immune cell-expressed VDR (22). While pharmacologic dosing of vitamin D has been similarly shown to inhibit DC

maturation and alloimmunity as well as augment the induction of Tregs through a cell-intrinsic, VDR-dependent mechanism (1, 15, 16, 23), previous work has not addressed the impact of immune cell-expressed VDR on alloimmunity in the context of physiological levels of vitamin D as we have done herein. Our findings newly indicate that costimulatory blockade-induced inhibition of alloimmunity, and associated prolonged allograft survival, do not require physiologic VDR signaling in immune cells. Because we did not observe differences in graft survival in our experiments between VDR deficient and WT chimeras, we did not specifically address whether the absence of immune cell VDR impacted Treg induction or function.

One limitation of BM chimera experiments, including experiments performed with VDR^{-/-} BM chimeras by others (21, 22), is that the reconstituted hosts contain a residual proportion (up to 15%) of host-derived, WT BM cells. As a consequence, in our model, residual WT host BM cells could theoretically inhibit the function of the majority VDR^{-/-} BM cells in the chimeras, prolonging graft survival and masking a potential adverse effect of the VDR^{-/-} immune cells on graft survival despite costimulatory blockade. However, our documentation that the responding, graft-reactive CD8⁺ T cells are predominantly donor-BM-derived in the WT and VDR^{-/-} chimeras (Fig 5) argues against this possibility. Moreover, T cell-intrinsic abnormalities were attributed to VDR deficiency in other models in which VDR^{-/-}→WT chimeras were employed, despite having achieved similar levels of BM chimerism to those observed herein (21, 22).

It is possible that VD interactions with alternative receptors in immune cells, including the 1,25D(3)-Membrane Activated Rapid Response to Steroids (1,25D3-MARRS) receptor (24, 25), could exert immunomodulatory effects that compensate for VDR-deficiency, but to our knowledge there are no data to support such a mechanism.

In summary, our findings support the conclusion that that BM cell expression of VDR is not required for costimulatory blockade-induced prolongation of cardiac allograft survival in mice. While VD-deficiency, which is common in kidney transplant candidates, likely contributes to a wide array of human immune and metabolic abnormalities, the results of the current work suggest that it is unlikely that VD deficiency will impair the efficacy of costimulatory blockade (including Food and Drug Association-approved CTLA4Ig) in human kidney transplant candidates.

Acknowledgments

The authors thank Paolo Cravedi, Anita Mehrotra, and Andrea Branch (Icahn School of Medicine at Mount Sinai) for their critical comments. The authors also thank the Microsurgery Shared Resource Facility at the Icahn School of Medicine at Mount Sinai for performing the heart transplant procedures and the histology core at the Icahn School of Medicine at Mount Sinai for assistance with H&E staining of graft tissue.

Funding: The work was supported by NIH grant AI43578 awarded to P Heeger.

Abbreviations

1,25(OH)₂D 1, 25-dihydroxyvitamin D

25(OH)D	25-hydroxyvitamin D
BM	Bone marrow
CFA	complete Freund's adjuvant
IFNγ	Interferon gamma
MST	median survival time
OVA	chicken egg ovalbumin
RXR	retinoid X receptor
Treg	regulatory T cell
VDR	Vitamin D receptor
WT	wild type

References

- McGregor R, Li G, Penny H, Lombardi G, Afzali B, Goldsmith DJ. Vitamin D in renal transplantation - from biological mechanisms to clinical benefits. *Am J Transplant*. 2014; 14:1259–1270. [PubMed: 24840071]
- Christakos S, Hewison M, Gardner DG, Wagner CL, Sergeev IN, Rutten E, et al. Vitamin D: beyond bone. *Ann N Y Acad Sci*. 2013; 1287:45–58. [PubMed: 23682710]
- Ooi JH, McDaniel KL, Weaver V, Cantorna MT. Murine CD8+ T cells but not macrophages express the vitamin D 1 α -hydroxylase. *J Nutr Biochem*. 2014; 25:58–65. [PubMed: 24314866]
- Joseph RW, Bayraktar UD, Kim TK, et al. Vitamin D receptor upregulation in alloreactive human T cells. *Hum Immunol*. 2012; 73:693–698. [PubMed: 22548720]
- van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D₃: basic concepts. *J Steroid Biochem Mol Biol*. 2005; 97:93–101. [PubMed: 16046118]
- Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006; 311:1770–1773. [PubMed: 16497887]
- Wang Q, Li H, Xie H, et al. 25-Hydroxyvitamin D₃ attenuates experimental periodontitis through downregulation of TLR4 and JAK1/STAT3 signaling in diabetic mice. *J Steroid Biochem Mol Biol*. 2013; 135:43–50. [PubMed: 23333931]
- Ooi JH, Li Y, Rogers CJ, Cantorna MT. Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate-induced colitis. *J Nutr*. 2013; 143:1679–1686. [PubMed: 23966330]
- Liu W, Chen Y, Golan MA, et al. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *J Clin Invest*. 2013; 123:3983–3996. [PubMed: 23945234]
- Na S, Ma Y, Zhao J, et al. A Nonsecosteroidal Vitamin D Receptor Modulator Ameliorates Experimental Autoimmune Encephalomyelitis without Causing Hypercalcemia. *Autoimmune Dis*. 2011; 2011:132958. [PubMed: 21318047]
- Driver JP, Lamont DJ, Gysemans C, Mathieu C, Serreze DV. Calcium insufficiency accelerates type 1 diabetes in vitamin D receptor-deficient nonobese diabetic (NOD) mice. *Endocrinology*. 2011; 152:4620–4629. [PubMed: 21952242]
- Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, Cantorna MT. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. *Mol Endocrinol*. 2003; 17:2386–2392. [PubMed: 14500760]
- Hartmann B, Heine G, Babina M, et al. Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response. *Allergy*. 2011; 66:540–548. [PubMed: 21121929]

14. Li B, Baylink DJ, Deb C, et al. 1,25-Dihydroxyvitamin D3 suppresses TLR8 expression and TLR8-mediated inflammatory responses in monocytes in vitro and experimental autoimmune encephalomyelitis in vivo. *PLoS One*. 2013; 8:e58808. [PubMed: 23516559]
15. Adorini L. Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting autoimmune diabetes. *Ann N Y Acad Sci*. 2003; 987:258–261. [PubMed: 12727648]
16. Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1 α ,25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. *Proc Natl Acad Sci U S A*. 2001; 98:6800–6805. [PubMed: 11371626]
17. Froicu M, Cantorna MT. Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. *BMC Immunol*. 2007; 8:5. [PubMed: 17397543]
18. Mathieu C, Van Etten E, Gysemans C, et al. In vitro and in vivo analysis of the immune system of vitamin D receptor knockout mice. *J Bone Miner Res*. 2001; 16:2057–2065. [PubMed: 11697802]
19. Kalache S, Lakhani P, Heeger PS. Effects of preexisting autoimmunity on heart graft prolongation after donor-specific transfusion and anti-CD154. *Transplantation*. 2014; 97:12–19. [PubMed: 24056628]
20. Valujskikh A, Pantenburg B, Heeger PS. Primed allospecific T cells prevent the effects of costimulatory blockade on prolonged cardiac allograft survival in mice. *Am J Transplant*. 2002; 2:501–509. [PubMed: 12118893]
21. Bruce D, Cantorna MT. Intrinsic requirement for the vitamin D receptor in the development of CD8 α α -expressing T cells. *J Immunol*. 2011; 186:2819–2825. [PubMed: 21270396]
22. Mayne CG, Spanier JA, Relland LM, Williams CB, Hayes CE. 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. *Eur J Immunol*. 2011; 41:822–832. [PubMed: 21287548]
23. Amuchastegui S, Daniel KC, Adorini L. Inhibition of acute and chronic allograft rejection in mouse models by BXL-628, a nonhypercalcemic vitamin D receptor agonist. *Transplantation*. 2005; 80:81–87. [PubMed: 16003237]
24. Buitrago C, Vazquez G, De Boland AR, Boland R. The vitamin D receptor mediates rapid changes in muscle protein tyrosine phosphorylation induced by 1,25(OH)(2)D(3). *Biochem Biophys Res Commun*. 2001; 289:1150–1156. [PubMed: 11741312]
25. Wu W, Beilhartz G, Roy Y, et al. Nuclear translocation of the 1,25D3-MARRS (membrane associated rapid response to steroids) receptor protein and NFkappaB in differentiating NB4 leukemia cells. *Exp Cell Res*. 2010; 316:1101–1108. [PubMed: 20064506]

Figure 1.

VDR-deficiency does not impair bone marrow reconstitution in chimeric animals. Representative flow cytometry plots (A) and quantified results from 4 mice per group (B) of donor (CD45.2) vs recipient (CD45.1) expression on peripheral blood cells 8 weeks after bone marrow transplantation. There were no significant differences between groups. C. Serum 25(OH)D levels in WT mice and in chimeras (p=ns among groups).

Figure 2. Similar T and B cell phenotypes in WT and VDR^{-/-} BM chimeras. Representative flow cytometry plots (A) and quantified percentages of 4 animals per group (B) documenting CD44 and CD62L expression patterns on CD4⁺ and CD8⁺ splenic T cells in chimeras > 8 weeks after BM transplantation. C. Percentages of B220⁺ B cells in spleens of chimeric animals and control WT mice (n=3 per group). There were no significant differences among groups for any of the parameters tested.

Figure 3.

Similar monocyte/DC phenotypes in WT and VDR^{-/-} BM chimeras. A. Representative flow cytometry plots depicting CD11c and CD11b expression patterns in spleen cells of chimeras > 8 weeks after BM transplantation. There were no significant differences between groups (n=3/group). B-C. Quantified class II MHC and CD86 expression on splenic CD11c/CD11b subsets, and on splenic B220+ B cells of WT and VDR^{-/-} BM chimeras >8 weeks after BM transplantation. There were no significant differences between groups for any of the parameters tested.

Figure 4.

BM cell VDR-deficiency does not impact cardiac allograft survival. A. Survival of bm12 hearts in WT B6 recipients and in WT and VDR^{-/-} BM chimeras (n=4–7 per group, p=ns among the 3 groups). B. Survival of MHC-disparate BALB/c heart grafts in WT and VDR^{-/-} BM chimeras that were untreated (black), or treated with a single dose of anti-CD154 mAb at 200 µg (blue) or 500 µg (red). Graft survival did not differ between the 2 sets of chimeric animals regardless of the treatment regimen (* p<0.05 vs untreated controls). C.

Survival of MHC-disparate BALB/c heart grafts in WT and VDR^{-/-} BM chimeras treated with a single dose of CTLA4Ig (p<0.05 between groups).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 5.

BM cell deficiency of VDR does not alter T cell immune responses. A. Representative flow cytometry plots depicting percentage of IFN γ -producers, gated on splenic CD8⁺ T cells obtained at rejection from chimeric recipients of allogeneic heart transplants (untreated). B-C. Quantified results from untreated (B) and CTLA4Ig-treated (C) recipients (n=3–4/group). No significant differences were observed. No IFN γ was detected in the CD4⁺ populations of CTLA4Ig treated animals (not shown). D. Representative flow cytometry plots depicting the percentage of donor (CD45.2) and host (CD45.1) CD8⁺ IFN γ -producers from the

experiment shown in 5C. * $p < 0.05$ vs. host cells. The experiment was repeated with similar results E. Alloantibody titers in chimeric recipients treated with CTLA4Ig at the time of rejection. No differences were noted between groups. F. Frequencies of OVA-specific, splenic IFN γ -producers (ELISPOT) in WT and VDR $^{-/-}$ chimeras, 14 d after immunization with OVA/CFA. No significant differences between groups.