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Effects of 1α,25(OH)$_2$D$_3$ and Its Analogs on Dendritic Cell Function

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Abstract 1α,25-Dihydroxyvitamin D$_3$ (1α,25(OH)$_2$D$_3$) and non-calcemic vitamin D analogs induce a persistent state of immaturity in dendritic cells both in vitro and in vivo. These effects are transcriptional in nature, involve alterations in surface ligands as well as cytokine synthesis and release, and are dependent upon the presence of the vitamin D receptor. The vitamin D endocrine system could also play a role in altering immune function in normal physiological conditions. Distinct differences exist in lymph node dendritic cells of vitamin D receptor null mutant mice when compared to normal mice. J. Cell. Biochem. 88: 323–326, 2003.

Key words: 1α,25-Dihydroxyvitamin D$_3$; dendritic cells; immune function

1α,25-Dihydroxyvitamin D$_3$ (1α,25(OH)$_2$D$_3$) plays an important role in the maintenance of calcium and phosphorus homeostasis [Kumar and Craig, 1997; Jones et al., 1998]. The effects of vitamin D in the intestine, as well as in bone, have been extensively studied in the past [Kumar and Craig, 1997; Jones et al., 1998; Gurlek and Kumar, 2001; Gurlek et al., 2002]. The mechanisms underlying many of these functions have also been delineated.

ROLE OF 1α,25(OH)$_2$D$_3$ IN MODULATION OF IMMUNE FUNCTION

1α,25(OH)$_2$D$_3$ has also been shown to play an important role in the modulation of immune function [Lemire, 1992; Mathieu and Adorini, 2002]. For example, 1α,25(OH)$_2$D$_3$ has been shown to inhibit T-cell activation and to inhibit or stimulate various T-cell subtypes [Lemire et al., 1985, 1995]. 1α,25(OH)$_2$D$_3$ also attenuates T-cell-mediated disease activity in experimental allergic encephalomyelitis [Lemire and Archer, 1991; Cantorna et al., 1996], collagen-induced arthritis [Cantorna et al., 1998], autoimmune thyroiditis [Fournier et al., 1990], hereditary diabetes [Mathieu et al., 1992; Adorini et al., 2002; Gregori et al., 2002], Heymann nephritis [Branisteanu et al., 1993], and transplant rejection [Veyron et al., 1993; Hullett et al., 1998].

DENDRITIC CELL FUNCTION AND THE REGULATION OF IMMUNE RESPONSE

The activation of T-cells by antigen presenting cells is an important mechanism by which immune function is modulated [Banchereau and Steinman, 1998; Palucka and Banchereau, 1999; Steinman, 1999]. Dendritic cells are a class of antigen presenting cells that potently stimulate CD4 (helper) T-cells and CD8 (cytolytic) T-cells. Small numbers of dendritic cells pulsed with low doses of antigen stimulate strong T-cell responses in naïve and quiescent T-cells. Immature dendritic cells, however, have weak in vitro immunostimulatory power and do not bring about potent immune sensitization in vivo (Table I). Hence, agents that maintain

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TABLE 1. Characteristics of Immature and Mature Dendritic Cells

Immature DC	Mature DC
High intracellular MHCII (MIICs)	High surface MHCII
Endocytosis, including FcR	Low endocytosis and FcR
Low CD54, 58, 80, 86	High CD54, 58, 80, 86
Low CD40, CD25, IL-12	High CD40, CD25, IL-12
Low CD83, p55	High CD83, p55
Low granule antigens	High M342, 2A1, MIDC-8 antigens
Actin cables	No actin cables

Modified from Banchereau and Steinman [1998].

dendritic cells in an immature state could be of use in the treatment of various immune diseases and in the prevention of rejection following transplantation.

EFFECT OF VITAMIN D ANALOGS ON DENDRITIC CELL FUNCTION

We examined the effects of $1\alpha,25(OH)_2D_3$ and a potent vitamin D analog ($1\alpha,25(OH)_2$ 19-ene, 23-yne, 19-nor-vitamin D_3) on dendritic cell function in vitro and in vivo [Griffin et al., 2000, 2001]. Our observations are consistent with those reported by other groups [Zhou et al., 1991; Wali et al., 1995; Pirro et al., 1997; Penna and Adorini, 2000]. We showed that the addition of a vitamin D analog to in vitro derived mouse dendritic cells resulted in a state of immaturity in these cells with low-level expression of cell surface markers of maturation [Griffin et al., 2000, 2001]. The yield of dendritic cells in culture was not significantly affected except at the highest doses of vitamin D analog used. The surface levels of both MHC II and co-stimulatory ligands in dendritic cells cultured from murine bone marrow in the presence of $1\alpha,25(OH)_2D_3$, or the related analog, were low, showing that these analogs caused a reduction in multiple markers of mature dendritic cells. We showed that the effect on combined surface levels of co-stimulatory molecules was dependent upon the presence of vitamin D receptor since vitamin D receptor null mutant (knockout (KO)) mice showed no such inhibition of cell surface marker expression. We also showed that vitamin D analog-treated dendritic cells failed to potently stimulate T-cell division in co-culture experiments. Thus, there is strong evidence showing that potent vitamin D analogs inhibit dendritic cell maturation and function in cell culture models.

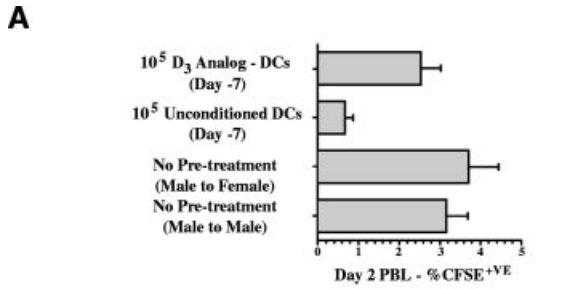
In further experiments, we showed that vitamin D treatment of dendritic cells inhibited increases in surface markers of maturation following treatment of dendritic cells with macrophage conditioned medium or lipopolysaccharide [Griffin et al., 2001]. We also demonstrated that dendritic cells secreted significantly less IL12 following treatment with vitamin D analog. TGFβ1 levels, however, did not change significantly.

In order to assess the effects of $1\alpha,25(OH)_2D_3$ and vitamin D analogs on dendritic cell function in vivo, we treated female mice with cultured male dendritic cells that had been exposed either to vitamin D analog or to vehicle [Griffin et al., 2001]. The ability to remove labeled male splenocytes from the circulation was tested in groups of animals that had either been pre-treated with vitamin D analog-DCs or vehicle-DCs and compared to non-pre-treated groups. As can be seen in Figure 1A, pretreatment with vehicle DCs resulted in accelerated clearance of infused male splenocytes in female mice in vivo, whereas pre-treatment with vitamin D analog-DCs did not. Furthermore, when transplantation of male skin was subsequently carried out, it was found that pre-treatment with vitamin D analog-DCs resulted in significantly prolonged graft survival compared to animals receiving no pre-treatment (Fig. 1B).

To determine whether lack of vitamin D receptor influences dendritic cell physiology in vivo, we examined subcutaneous lymph nodes from wild type (WT) or vitamin D receptor KO mice. As seen in Figure 2, the lymph nodes from vitamin D receptor KO mice were larger than those from WT animals. Analysis of lymph node cells from WT or vitamin D receptor KO mice showed that the proportion of highly mature dendritic cells in lymph nodes was higher in the KO animals than in the WT animals. Lymph node dendritic cells from KO animals had higher cell surface levels of the maturation markers MHC II, CD40, CD80/CD86 than did dendritic cells of KO animals [Griffin et al., 2001]. Similar effects were not noted in the spleen.

CONCLUSIONS

Vitamin D induces a state of dendritic cell immaturity in vitro and in vivo. The vitamin D



B

		PBL %CFSE^{+ve} (Day 16 Post Splenocyte Graft)	Day of Skin Graft Rejection
D_3 Analog-Conditioned DC Pre-inoculation (n=10)	1	2.47	> 100
	2	2.34	> 100
	3	1.76	> 100
	4	0.70	> 100
	5	0.53	> 100
	6	0.49	> 100
	7	0.10	27
	8	0.00	22
	9	0.00	12
	10	0.00	11
No Pre-inoculation	(n = 5)	0.12 ± 0.03	17 ± 2

Fig. 1. **A**: A group of five male B6 and three groups of five female B6 mice were inoculated with CFSE-labeled male B6 splenocytes. Two days later grafted cells were quantified. Results are expressed as mean ± SD for each group. Male and female recipients that had received no pretreatment had 3.15 ± 0.54% and 3.70 ± 0.75% CFSE+ve cells, respectively ($P = 0.2$). Females inoculated 7 days previously with 105 unconditioned male B6 DCs had 0.67 ± 0.20% CFSE+ve cells ($P = 0.0002$ compared with male to male group). Females preinoculated with 105 D3 analog-conditioned DCs had 2.54 ± 0.50% CFSE+ve cells ($P = 0.1$ compared with male to male group). **B**: Ten female B6 mice received 105 D3 analog-conditioned male B6 DCs followed 7 days later by CFSE-labeled male B6 splenocytes. A control group of five female B6 mice received labeled splenocytes without DC preinoculation. The percent of CFSE+ve PBLs 16 days later was determined in each animal (center). Six of ten animals preinoculated with D3 analog-conditioned DCs had levels of labeled cells greater than the control group. Seven days later all animals were grafted with male B6 skin and time to graft rejection was determined (right). The control group rejected male skin grafts between 15 and 19 days posttransplant. Six of ten DC-treated animals retained skin grafts indefinitely (>100 days), whereas, the remaining four rejected grafts between 11 and 27 days posttransplant. Indefinite skin graft survival correlated with prolonged retention of male splenocytes in the group preinoculated with D3 analog-conditioned DCs [Griffin et al., 2001].

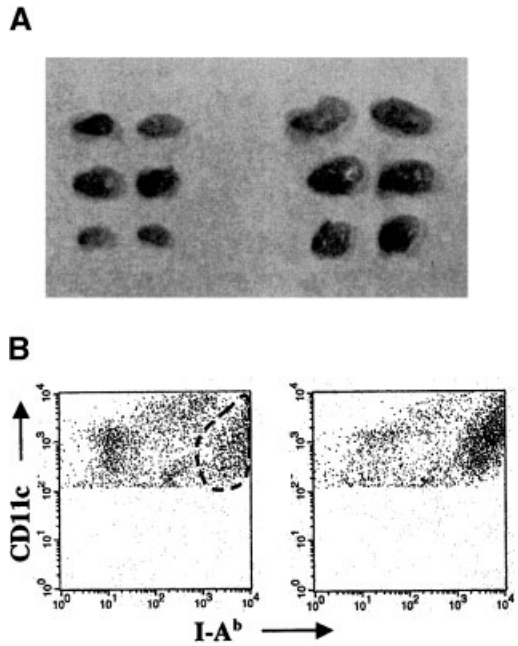


Fig. 2. Examination of subcutaneous lymph nodes from VDR wild type (WT) and VDR knockout (KO) mice. **A**: Lymph nodes from KO mice were uniformly larger than those of WT (representative example from one pair of animals). **B**: An example is shown of flow cytometric analysis of lymph node cells from a VDR WT and KO pair. Class II MHC (I-A^b) expression by CD11c^{+ve} dendritic cells is shown. Within the CD11c^{+ve} cells from both animals populations with low, intermediate, and high expression of I-A^b are present. For the KO animal the I-A^b high cells (encircled) represent the predominant population with relative reductions in the I-A^b low and intermediate cells compared with the WT animal (modified from Griffin et al., 2001).

receptor appears to be essential for the effects of vitamin D analogs on dendritic cell function. Vitamin D receptor KO mice have enlarged subcutaneous lymph nodes and changes in dendritic cell maturation consistent with changes observed following addition of vitamin D metabolites in dendritic cell cultures. Potent noncalcemic vitamin analogs should find use in altering immune function in a number of clinically important situations.

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