In Vivo Effects of Calcitriol on Phenotypic and Functional Properties of Dendritic Cells

Mohammad Mahdi Eftekharian[1], Amir Hassan Zarnani[2,3], Seyed-Mohammad Moazzeni[1*]

[1]Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, [2]Nanotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran, [3]Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: Dendritic cells (DCs) play a central role in the initiation and expansion of T cell mediated immune responses with potential immunotherapy application. The compounds which have the ability to induce immunomodulatory effects on DCs may be employed for the treatment of immunopathologic conditions such as autoimmune diseases. **Objective:** The aim of this study was to investigate the in vivo effects of calcitriol (active form of vitamin D3) on DCs. **Methods:** 0.1 microgram calcitriol was injected intra-peritoneally into C57BL/6 mice every other day within 3 weeks, and spleen DCs were extracted by magnetic beads. The phenotypic and functional properties of DCs were studied by flow cytometry and mixed lymphocyte reaction (MLR), respectively. **Results:** The expression of CD86 and MHC II, as maturation markers and costimulatory molecules were significantly decreased ($p=0.028$ and $p=0.047$, respectively) while CD11b expression, as a marker of mice myeloid DCs which mostly induces Th2 cytokine profile, was significantly increased ($p=0.011$). Allogeneic T cell stimulation in MLR was also significantly inhibited in comparison with the control groups ($p<0.05$). **Conclusion:** Our data indicate that in vivo calcitriol administration inhibits maturation and activation of DCs in the same manner as in vitro conditions.

Keywords: Cholecalciferol, Dendritic cells, Immunomodulation, Vitamin D3

INTRODUCTION

As antigen processors, DCs possess an important role in the management of immune response. Based on this fact, scientists are aiming at manipulation of the immune response towards preferred directions, using compounds which alter the functional properties of these crucial cells. Both in mice and human, there are two subtypes of dendritic cells, conventional (cDC) and plasmacytoid DCs (pDC) (1,2).

*Corresponding author: Dr. Seyed-Mohammad Moazzeni, Department of Immunology, Faculty of Medical Sciences, Tarbiat modares University, Tehran, Iran, Tel: (+) 98 21 82883846, Fax: (+) 98 21 82883846, e-mail: Moazzeni@modares.ac.ir

Spleen DCs are recognized by the expression of CD11c as a common marker for all subsets of these cells while on the basis of CD4, CD8α and CD11b expression, three subtypes of cDCs have been identified in this organ: $CD4^+$ $CD8\alpha^-$ $CD11b^+$ (Myeloid) and $CD8\alpha^+$ $CD4^-$ $CD11b^-$ (Lymphoid) DCs that localize mostly in the marginal zone and the T-cell zone, respectively. $CD4^-$ $CD8\alpha^-$ $CD11b^+$ DCs have also been identified and are called double-negative DCs (2,3). DC subtypes have different and in some aspects partially opposed phenotypic and functional properties. Lymphoid DCs in mice after antigen presentation and secretion of high amounts of IL-12 and IFN-γ, lead immune response towards Th1 pathway, whereas myeloid ones shift immune function to Th2 type and produce IL-4 and IL-10 cytokines (in contrast to human DC subpopulations). It is evident that induction of Th2 pathway by an appropriate DC subtype can be effective and useful in prevention and treatment of Th1-dependent autoimmune diseases, such as multiple sclerosis (4-8).

1,25 Dihydroxycholecalciferol (calcitriol), the active form of vitamin D3, has a crucial physiologic and hormonal role in the body. Although vitamin D3 is provided through diet but it is mostly made by the skin through the action of light. Exposure of skin to ultraviolet (UV) light causes the first step of vitamin D3 production. In this stage 7-dehydrocholestrol is converted to pre-vitamin D3, and then vitamin D3 is produced spontaneously through a temperature dependent reaction. To gain biologically active form of vitamin D3, this compound must be hydroxylated in the liver and kidney by D3-25-hydroxylase and 25 OHD3-1-α-hydroxylase, respectively, resulting in production of calcitriol as the active form of this vitamin. Apart from this classical metabolic pathway, several cells such as monocytes/macrophages, pneumocytes and keratinocytes can synthesize calcitriol (9-12).

Calcitriol and its synthetic analogues such as TX527 have regulatory effects on immune system and are signified as immunomodulatory agents. These regulatory effects are exerted after attachment of these compounds to their specific receptors (Vitamin D Receptor = VDR), a member of the superfamily of nuclear hormone receptors, on target cells such as T, B, NK cells, DCs etc. (9,12-16). Furthermore it has been shown that VDR is constitutively expressed on antigen presenting cells but is also inducible by lymphocytes (9-14). Calcitriol reduces the production of Th1-dependent and inflammatory cytokines such as IL-1, IL-2, IL-6, IL-12, TNF-α and IFN-γ downstream of its effects on T cells and monocytes. Similar in vitro effects on DCs are reported experiments as well (9, 16-19). Because in vivo cellular interactions and microenvironmental conditions are completely different from in vitro ones and considering the importance of these critical determinants in the course of immune response, it seems that we cannot generalize the in vitro observations to in vivo conditions. Therefore, we decided to investigate the in vivo effects of calcitriol on DC phenotype and function.

MATERIALS AND METHODS

Animals. 6-8 weeks old C57BL/6 female mice were obtained from Pasteur Institute of Iran. Mice were kept under optimal conditions of hygiene, temperature, and humidity with 12 hours of light and 12 hours of darkness cycle and were allowed food and water ad libitum. All experimental procedures on animals were approved by the ethical committee of Faculty of Medical Sciences, Tarbiat Modares University.

Treatments. 0.1 microgram calcitriol (Sigma, USA) was injected intra-peritoneally every other day within 3 weeks (20,21). The mice in the control group were treated with 2% ethanol in PBS as vehicle. Each group consisted of 6 age-matched female mice.

Purification of Splenic DCs. This step was done according to our previous report (22) and using cell separation columns according to manufacturer's instruction. Briefly, spleens of the control and test mice were isolated and minced to very small pieces. The spleen tissues were suspended in cold (4˚C) PBS containing 1.2 mg/ml collagenase D (Roche, Germany). After incubation at 37˚C for 40 minutes, the collagenase was inactivated by EDTA and the cells suspension was passed through a cell mesh and the collected cells were washed twice. Mononuclear cells (MNC) were then separated by Nycodenz (Axis-shield, Norway); a material used in density gradient cell separation techniques. The MNCs were then washed three times and incubated for 25 minutes at 4°C with 2.5% mice serum to block the non-specific binding sites. Anti-CD11c magnetic beads (Miltenyi Biotech, Germany) were added to the cells and incubated at 4°C for 30 minutes and the non-bonded antibodies were washed out. The minimacs separator (MS) cell columns were put into the magnetic field (MS separation unit, Miltenyi Biotech, USA) and washed by 1.5 ml EDTA-containing PBS.

The cell suspension was then passed through the column to absorb the magnetic bead coated DCs and the unwanted cells were washed out using at least 3 washes of 500 µl EDTA-containing PBS. The isolated DCs were collected by disconnecting the column from the magnetic field and injecting it with 1 ml PBS-EDTA under pressure.

Two-Color Flow Cytometric Analysis. The expression of phenotypic and maturation markers on the isolated dendritic cells was analyzed by flow cytometry. Simply, 1 µl of FITC conjugated anti- CD8α, CD11b, MHC II, CD40 and CD86 (BD biosciences, USA) or relevant isotype controls, was added to separate flow cytometry tubes containing 5×10^4 cells. After 15 min incubation on ice and in dark, 1 µl of PE conjugated anti-CD11c (BD biosciences, USA) was added to all the tubes which were incubated under the same conditions for another 30 minutes.

The cells were then washed by PBS and fixed by 2% paraformaldehyde before flow cytometric analysis by FACScan flow cytometer (BD biosciences, USA). The results were analyzed by WinMDI software. Purity of isolated DC's was confirmed by staining them only with PE conjugated anti-CD11c. The cells stained with appropriate FITC or PE conjugated irrelevant antibodies were used as isotype controls.

Mixed Lymphocyte Reaction (MLR). 1.5×10^4 separated and γ-irradiated (3000 rad) DCs were cultured in triplicate in 96-well plates (Nunc, Denmark) together with 1.5×10^5 allogeneic T cells. The T cells were obtained and purified by passage of a single cell suspension of BALB/c mice lymph nodes over a nylon wool column. The purity of T cell preparations were determined by flow cytometry as $CD3^+$ cells and were routinely more than 90%. After 54 hours of incubation at 37°C and 5% CO2, 1 µCi ^{3}H-Thymidine (Amersham Biosciences, UK) was added to all wells. On the 3rd day of culture, the cells were harvested on glass fiber filter papers and their proliferation was measured in a liquid scintillation counter (Wallac, Pharmacia, Sweden). Results were expressed as the mean count per minutes (CPM) of the triplicate cultures. T cells cultured in the presence of PHA as a polyclonal activator and DC or T cells alone were considered as positive and negative controls, respectively.

Statistical Analysis: Results are presented as Mean ± SD of at least six different experiments. Mann-Whitney test was used for the evaluation of statistical differences between the results and p values less than 0.05 were considered statistically significant.

RESULTS

Separation of DCs. DCs were separated from the spleens of C57BL/6 mice through enzyme digestion followed by positive selection using anti-CD11c coated magnetic beads. The purity of DCs was determined as 91.28 ± 1.11% of separated cells using flow cytomety (Figure 1).

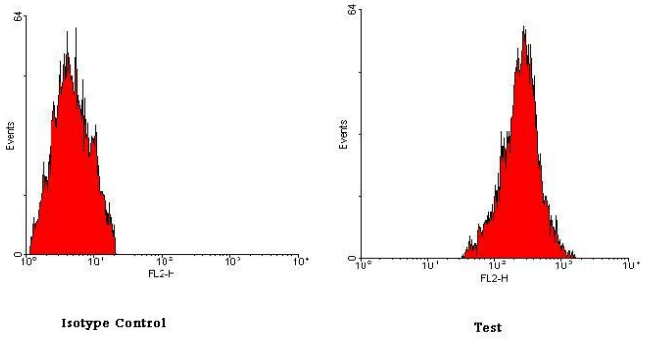

Figure 1. Flowcytometric analysis of DCs. PE conjugated anti-CD11c staining and flowcytometry was used for determining the purity of isolated DCs. The histogram shows a representative of 12 separate experiments.

Immunophenotypic Studies. The percentage of maturation and the co-stimulatory markers-bearing DCs was determined by two color flow cytometry. The expression density of studied molecules was also verified as mean fluorescent intensity (MFI). In vivo calcitriol-treated DCs, showed reduced percentages of positive cells and particularly lower expression of CD40, CD86, and MHC class II as maturation markers and lower expression of CD8α as a marker for lymphoid subsets of DCs which induce Th1 responses in mice, while CD11b expression which is considered as a marker of mouse myeloid DCs that mostly induce Th2 responses, was increased (Figures 2 and 3).

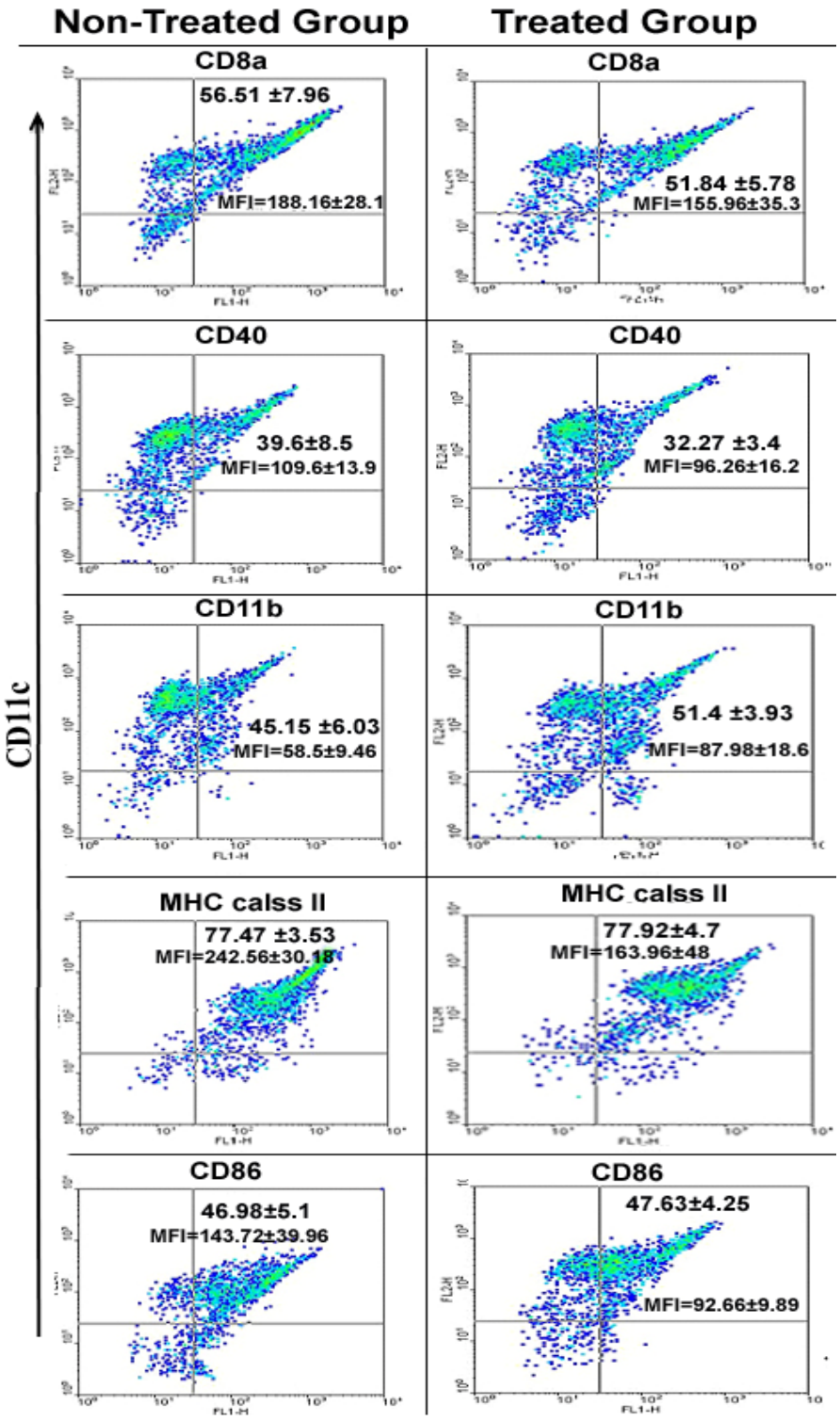

Figure 2. Immunophenotyping of calcitriol treated DCs. Mice were treated with calcitriol for three weeks. Dendritic cells were separated from calcitriol treated and non-treated mice and the expression of subtypes and maturation markers were determined on CD11c+ DCs. The percentage of positive cells and Mean Fluorescent Intensity (MFI) of studied markers has been shown as Mean ± SD of 6 independent experiments.

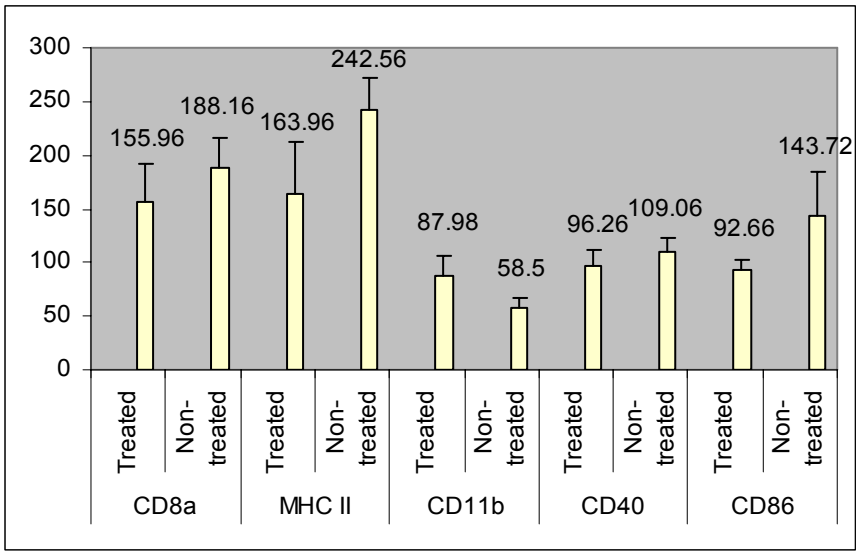

Figure 3. The expression intensity of studied molecules on calcitriol treated and non-treated DCs. The density of subtypes and maturation markers on DCs was determined as Mean Fluorescent Intensity (MFI). The histograms show the MFI of surface markers as Mean and SD of six independent experiments in each group.
(P values: CD8α=0.117, CD40=0.251, CD11b=0.011, MHC class II=0.047 and CD86=0.028)

Statistical analysis of the results showed that in vivo treatment by calcitriol does not affect the percentage of marker-bearing cells significantly ($p>0.05$), whereas it causes a significant ($p<0.05$) decrease in the expression level (MFI) of all markers studied except CD8α and CD40. Furthermore spleen size was smaller in calcitriol treated mice compared with the control group (data not shown).

Mixed Leukocyte Reaction (MLR). We attempted to investigate the immunostimulatory potential of isolated DCs by standard MLR test. Gamma irradiated DCs from calcitriol treated and untreated mice were co-cultured with allogeneic T cells. The T cell stimulation and proliferation was measured through ^{3}H-Thymidine incorporation. Statistical analysis indicated a significant ($p=0.004$) decrease in the T cell stimulation potential of DCs from calcitriol treated mice (CPM = 25397 ± 855) in comparison with DCs from untreated mice (CPM = 31921 ± 1289).

DISCUSSION

Several effects on immune cells as well as on DCs have been reported for calcitriol; the natural active form of vitamin D3 (9,12-16). Indeed, almost all studies on the effects of vitamin D3 on DCs were performed in vitro (9,16-19,23,24). In this study, we tried to address the in vivo effects of calcitriol on DCs. Our results showed that although the changes in the relative percentage of the marker-positive DCs, after their in vivo treatments by calcitriol compared with untreated DCs, were not statistically significant, the expression of most markers on the cell surface was significantly changed. In fact in vivo calcitriol treated DCs showed lower state of maturation which appeared as lower ex-

pression of their maturation markers of CD40, CD86, and MHC II. However, the decrease in CD40 expression was not statistically significant.

Moreover, calcitriol treated DCs showed a lower ability for T cell stimulation than the non-treated cells. In fact, the CPM of allogenic MLR in calcitriol treated groups (25397 ± 855) was significantly lower than the untreated controls (31921 ± 1289).

Consistent to our findings Penna et al. (11) and Berer et al. (9) reported that human DCs are amongst the major targets for in vitro induced immunosuppressive activity of calcitriol. They showed that the presence of calcitriol during LPS-induced maturation of monocyte derived DCs caused downregulation in DC maturation which appeard as a high CD1a and a low CD83 expression. Moreover, the expression of costimulatory molecules CD40, CD80, CD86 and MHC II was inhibited. They also demonstrated that calcitriol has a suppressive effect on the human DC allostimulation of T cells in allogenic MLR. This effect was accompanied by a decrease in IL-12 production and an increased secretion of IL-10.

Van Halteren et al. (25) and Gauzzi et al. (26), also studied the in vitro effects of TX527, a vitamin D3 analogue, on DC differentiation, maturation and function. Continuous addition of TX527 to the cell culture impaired IL-4 and GM-CSF induced DC differentiation as well as LPS and IFN-γ induced DC maturation. They demonstrated that these events also affect T cell function and cytokine production after encountering TX527 treated DCs. A synergic suppressive effect of calcitriol and dexamethasone on in vitro LPS-induced maturation of DCs was also reported (27).

Vitamin D exerts its effects on immune cells through its specific receptor (VDR). Vitamin D-binding protein (DBP), a serum globulin, which is mainly produced by the liver, transports vitamin D metabolites to target tissues and cells. It also helps to regulate the bioavailability of calcitriol. On the target cell, calcitriol is released from DBP, enters the cell, interacts with VDR and shuttles from cytoplasm to the nucleus. Nuclear VDR is a ligand-activated transcription factor which is activated by calcitriol. Activated VDR finally activates the target genes. Calcitriol thus exerts its biological actions through VDR-mediated gene regulation (11,12,23,28,29). Gauzzi et al. (30) showed the central role of interferon regulatory factor 4 (IRF-4), which is a lymphoid and myeloid restricted transcription factor of the IRF family, in calcitriol-mediated immunomodulation of DCs. Also it was revealed that calcitriol increases the expression of immunoglobulin-like transcript 3 (ILT3) on human DCs three to six fold. This finding suggests the involvement of ILT3 in the immunoregulatory properties of this active form of vitamin D3. Expression of ILT3 is associated with the induction of regulatory T cells which is crucial in the treatment and prevention of autoimmune diseases (19, 31).

Griffin et al. (32) used VDR knockout mice to study the importance of VDR in DC maturation. They showed that DC modulation by a synthetic analog of calcitriol is VDR dependent and causes a persistent state of immaturity. They reported that compared with intact animals, VDR-deficient mice had hypertrophy of the subcutaneous lymph nodes and an increase in the number of mature DCs in the lymph nodes and not in the spleen. However we observed that IP injection of calcitriol affects both the size of the spleen and maturation of its DCs.

DCs can either upregulate or downregulate the immune response depending on their subtype, degree of maturation and microenvironment. Induction of T cell response requires both T cell receptor activation and costimulation (27). Hence a decrease in the expression of costimulatory molecules, which is accompanied by an immature state of DCs, reduces their T cell stimulatory potential. Our results which was consistent with in

vitro reports showed that calcitriol downregulates the expression of costimulatory molecules on DCs. Therefore, the significant decrease in T cell activation rate which was observed in MLR reaction following calcitriol-treatement of DCs was expectable. Gregori et al. (33) also orally administered mycophenolate mofetil in combination with 1, 25-Dihydroxyvitamin D3 to mice in a transplantation model. They also showed the down-regulation of costimulatory molecules in DCs from the graft area along with an induction of regulatory T cells.

We also showed that CD8α expression, as a marker for lymphoid subsets of DCs which induce Th1 responses in mice, was downregulated, while CD11b expression, which is considered as a marker of Th2 inducing DCs, was increased.

Dendritic cells are a heterogeneous population of immunocytes with diverse phenotypes and functions. Both in human and mice DCs are usually subdivided into at least two subtypes, based on their tissue localization and expression of surface markers (2,3). These subtypes of DCs are functionally distinct as well. In mice, lymphoid DCs ($CD8\alpha^+$, $CD11b^-$) direct immune response towards Th1 pathway after antigen presentation and secretion of high amount of IL-12 and IFN-γ. However, myeloid DC's ($CD8\alpha^-$ and $CD11b^+$) shift the immune function towards Th2 type and produce IL-4 and IL-10 cytokines (4-8).

Penna et al. (34) reported that calcitriol selectively affects myeloid DCs in humans. Based on their reports, after exposure of human DCs to calcitriol, the production of IL-12 from myeloid DCs decreased and caused a significant reduction in their capacity to induce Th1 cells. Considering that human myeloid DCs are counterparts of mice lymphoid DCs, the work of Penna et al. is consistent with our findings, meaning that in both human and mice calcitriol shifts immune response toward a Th2 cytokine profile.

In conclusion, calcitriol has several in vivo inhibitory effects on DC maturation and expression of costimulatory molecules. Also this compound induces an immune shift toward Th2 cytokine profile. In fact the in vivo effects of calcitriol on DCs are comparable with its in vitro effects.

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REFERENCES:

1 Jacobs B, Wuttke M, Papewalis C, Seissler J, Schott M. Dendritic Cell Subtypes and In Vitro Generation of Dendritic Cells. Horm Metab Res. 2008; 40:99-107.
2 Merad M, Manz M G. Dendritic cell homeostasis. Blood. 2009; 113:3418-27.
3 Onji M, Akbar SMF. Dendritic Cells in Clinics. 2nd ed. Japan: Springer; 2008.
4 Jung S. Good, bad and beautiful-the role of dendritic cells in autoimmunity. Autoimmun Rev. 2004; 3:45-60.
5 Dakic A, Wu L. Hemopoietic precursors and development of dendritic cell populations. Leuk Lymphoma. 2003; 44: 1469-75.
6 Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol. 2002; 2: 151-61.
7 Hochrein H, O'keeffe M, Wagner H. Human and mouse plasmacytoid dendritic cells. Hum Immunol. 2002; 63:1103-10.
8 Ardavin C. Origin, precursors and differentiation of mouse dendritic cells. Nat Rev Immunol. 2003; 3:582-90.
9 Berer A, Stockl J, Majdic O, Wagner T, Kollars M, Lechner K, et al. 1,25-Dihydroxyvitamin D3 inhibits dendritic cell differentiation and maturation in vitro. Exp Hematol. 2000; 28:575-83.
10 Kreutz M, Andreesen R, Krause SW, Szabo A, Ritz E, Reichel H. 1,25-dihydroxyvitamin D3 production and vitamin D3 receptor expression are developmentally regulated during differentiation of human monocytes into macrophages. Blood. 1993; 82:1300-7.
11 Penna G, Adorini L. 1α,25-Dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol. 2000; 164:2405-11.

12 Van Etten E, Mathieu C. Immunoregulation by 1, 25-dihydroxyvitamin D3: Basic concepts. J Steroid Biochem Mol Biol. 2005; 97:93-101.
13 Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res. 1998; 13:325-49.
14 Adorini L, Penna G, Giarratana N, Roncari A, Amuchastegui S, Daniel KC, et al. Dendritic cells as key targets for immunomodulation by vitamin D receptor ligands. J Ster Biochem Mol Biol. 2004; 89-90:437-41.
15 Adorini L. Immunomodulatory effects of vitamin D receptor ligands in autoimmune diseases. Int Immunopharmacol. 2002; 2:1017-28.
16 Griffin MD, Kumar R. Effects of 1α,25(OH)2D3 and its analogs on dendritic cell function. J Cell Biochem. 2003; 88:323-26.
17 Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D3 analogs as immunomodulatory agents. Trends Mol Med. 2002; 8:174-9.
18 Enioutina EY, Bareyan D, Daynes RA. Vitamin D3-mediated alteration to myeloid dendritic cell trafficking in vivo expand the scope of their antigen presenting properties. Vaccine. 2007; 25:1236-49.
19 Adorini L, Giarratana N, Penna G. Pharmacological induction of tolerogenic dendritic cells and regulatory T cells. Semin Immunol. 2004; 16:127-34.
20 Muthian G, Raikwar HP, Rajasingh J, Bright JJ. 1,25-Dihydroxyvitamin D3 modulates JAK-STAT pathway in IL-12/IFNgamma axis leading to Th1 response in experimental allergic encephalomyelitis. J Neurosci Res. 2006; 83:1299-309.
21 Lemire JM, Archer DC. 1,25-Dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. J Clin Invest. 1991; 87:1103-7.
22 Zarnani AH, Moazzeni SM, Shokri F, Salehnia M, Dokouhaki P, Shojaeian J, et al. The efficient isolation of murine splenic dendritic cells and their cytochemical features. Histochem Cell Biol. 2006; 126:275-82.
23 Shimazaki M, Miyamoto Y, Yamamoto K, Yamada S, Takami M, Shinki T, et al. Analogs of 1α,25-dihydroxyvitamin D3 with high potency in induction of osteoclastogenesis and prevention of dendritic cell differentiation: Synthesis and biological evaluation of 2-substituted 19-norvitamin D analogs. Bioorgan Med Chem. 2006; 14:4645-56.
24 Piemonti L, Monti P, Sironi M, Fraticelli P, Leone BE, Dal Cin E, et al. Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. J Immunol. 2000; 164:4443-51.
25 Van Halteren AGS, Van Etten E, De Jong EC, Bouillon R, Roep BO, Mathieu C. Redirection of human autoreactive T-cells upon interaction with dendritic cells modulated by TX527, an analog of 1,25 dihydroxyvitamin D3. Diabetes. 2002; 51:2119-25.
26 Gauzzi MC, Purificato C, Donato K, Jin Y, Wang L, Daniel KC, et al. Suppressive effect of 1α,25-didydroxyvitamin D3 on type I IFN-Mediated monocyte differentiation into dendritic cells: Impairment of functional activities and chemotaxis. J Immunol. 2005; 174:270-6.
27 Pedersen AE, Gad M, Walter MR, Claesson MH. Induction of regulatory dendritic cells by dexamethasone and 1α,25-dihydroxyvitamin D3. Immunol Lett. 2004; 91:63-9.
28 VanAmerongen BM, Dijkstra CD, Lips P, Polman CH. Multiple sclerosis and vitamin D: an update. Eur J Clin Nutr. 2004; 58:1095-109.
29 Szeles L, Keresztes G, Torocsik D, Balajthy Z, Krenacs L, Poliska S, et al. 1,25-dihydroxyvitamin D3 is an autonomous regulator of the transcriptional changes leading to a tolerogenic dendritic cell phenotype. J Immunol. 2009; 182:2074-83.
30 Gauzzi MC, Purificato C, Conti L, Adorini L, Belardelli F, Gessani S. IRF-4 expression in the human myeloid lineage: up-regulation during dendritic cell differentiation and inhibition by 1α,25-dihydroxyvitamin D3. J Leukoc Biol. 2005; 77:944-7.
31 Penna G, Roncari A, Amuchastegui S, Daniel KC, Berti E, Colonna M, et al. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+ Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3. Blood. 2005; 106.:3490-7.
32 Griffin MD, Lutz W, Phan Vy A, Bachman LA, Mckean DJ, Kumar R. Dendritic cell modulation by 1α,25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a prsistent state of immaturity in vitro and in vivo. Proc Natl Acad Sci U S A. 2001; 98:6800-5.
33 Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM, Adorini L. Regulatory T cells induced by1{alpha},25-Dihydroxyvitamin D3 and Mycophenolate Mofetil Treatment Mediate Transplantation Tolerance. J Immunol. 2001; 167:1945-53.
34 Penna G, Amuchastegui S, Giarratana N, Daniel KC, Vulcano M, Sozzani S, et al. 1,25-Dihydroxyvitamin D3 selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells. J Immunol. 2007; 178:145-53.