Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 449–452
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Vitamin D and 1,25-dihydroxyvitamin D_3 as modulators in the immune system☆

Chantal Mathieu*, Evelyne van Etten, Brigitte Decallonne, Annapaula Guilietti, Conny Gysemans, Roger Bouillon, Lut Overbergh

LEGENDO, Katholieke Universiteit Leuven, O&N, Herestraat 49, 3000 Leuven, Belgium

Abstract

Treatment from weaning until old age with 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) prevents diabetes in NOD mice. It is mainly through its actions on dendritic cells (DCs), that $1,25(OH)_2D_3$ changes the function of potentially autoreactive T lymphocytes. In contrast, early life treatment (from 3 to 70 days of age) of NOD mice with vitamin D or $1,25(OH)_2D_3$ did not influence final diabetes incidence at 200 days of age. Also in spontaneous diabetic BB rats, diabetes could not be prevented by early life treatment (from 3 to 50 days of age) with vitamin D (1000 IU per day) or $1,25(OH)_2D_3$ (0.2 μg/kg per day or 1 μg/kg per 2 days). However, when NOD mice were made vitamin D deficient in early life (until 100 days of age), diabetes onset occurred earlier and final incidence was increased. These data further support a role for vitamin D and its metabolites in the pathogenesis of type 1 diabetes in NOD mice.

Keywords: Vitamin D; $1,25(OH)_2D_3$; NOD mice; Diabetes

1. Introduction

Vitamin D, and especially its activated form 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), besides displaying effects in calcium and bone metabolism, has potent effects on cell proliferation and differentiation in normal as well as in malignant cell types. Receptors for $1,25(OH)_2D_3$ have been found in different cells of the immune system [1]. The immune effects of $1,25(OH)_2D_3$ are mainly mediated through its action on antigen-presenting cells of which the dendritic cells (DCs) are the most potent population [2–4]. By in vitro treatment of DCs with $1,25(OH)_2D_3$, antigen-presentation is inhibited and the surface expression of costimulatory molecules as well as the production and secretion of IL-12 is down-regulated. Although direct effects on T lymphocytes have also been observed, it is mainly by this indirect pathway that $1,25(OH)_2D_3$ modulates the fate and function of $CD4^+$ T lymphocytes. Proliferation of T lymphocytes is inhibited and the panel of cytokines produced is modulated, shifting the phenotype towards a more regulatory T lymphocyte. These in vitro effects are reflected in vivo by the potential to prevent autoimmune diseases in different animal models and to prolong graft survival [5].

2. Prevention of autoimmune diabetes by $1,25(OH)_2D_3$ in NOD mice

$1,25(OH)_2D_3$ is able to prevent spontaneous autoimmune diabetes in NOD mice [6]. By life long treatment, being from weaning until old age, not only diabetes but also insulitis, the histological lesion preceding overt diabetes, can be partially prevented [7]. Among the immune disregulations described in NOD mice possibly leading to beta cell destruction, are the phenotypical and functional abnormalities of DCs [8–10]. In this study we investigated the effects of in vivo $1,25(OH)_2D_3$ treatment on DCs of NOD mice, isolated from bone marrow and thymus, in comparison with congenic non-diabetic NOR mice.

For this purpose female NOR and NOD mice were treated with $1,25(OH)_2D_3$ (obtained from J.P. Vandevelde, Duphar, Weesp, The Netherlands) at a concentration of 5 μg/kg three times weekly from weaning until 10 weeks of age. Vehicle treated age-matched female mice of the same strain served as controls. At 10 weeks of age, cells were harvested from bone marrow and thymus and DCs were purified by positive magnetic cell sorting (MACS) with CD11c-MircoBeads (Miltenyi Biotec, Auburn, CA, USA). $CD11c^+$ DCs from

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

* Corresponding author. Tel.: +32-16-346023; fax: +32-16-345934.
E-mail address: chantal.mathieu@med.kuleuven.ac.be (C. Mathieu).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.014

Table 1
The effects of in vivo $1,25(OH)_2D_3$ treatment on the surface expression of MHC II, CD86, CD40 and CD54 on $CD11c^+$ DCs purified from bone marrow of NOR and NOD mice

	NOR		NOD	
	Vehicle	$1,25(OH)_2D_3$	Vehicle	$1,25(OH)_2D_3$
MHC II (MFI)[a]	55 ± 44	24 ± 3	32 ± 29	29 ± 29
CD86 (MFI)	6.6 ± 2.5	4.9 ± 0.3	6.3 ± 3.0	9.3 ± 7.5
CD40 (MFI)	37 ± 9	41 ± 35	56 ± 49	82 ± 85
CD54 (%)[b]	0.20 ± 0.14	0.02 ± 0.01	0.51 ± 0.43	0.59 ± 0.67

Mice were vehicle-treated or $1,25(OH)_2D_3$-treated (5 μg/kg three times weekly) from weaning to 10 weeks of age. Bone marrow cells were harvested and $CD11c^+$ DCs were purified by MACS and analyzed by FACS for surface expression of MHC II, CD86, CD40 and CD54 within the $CD11c^+$ window. The results are expressed as mean ± S.D.

[a] Values for MHC II, CD86 and CD40 were expressed as mean fluorescence intensity (MFI).

[b] Values for CD54 were expressed as % CD54 high positive cells.

bone marrow and thymus, representing only about 1% of total bone marrow and thymus, obtained with a purity of 54 ± 18% and 15 ± 3%, respectively, were analyzed by fluorescence-activated cell sorting (FACS) for their surface expression of MHC II, CD86, CD40 and CD54 within the $CD11c^+$ window (Becton Dickinson, San Jose, CA, USA). For none of the surface markers investigated on DCs purified from bone marrow, significant differences could be observed between $1,25(OH)_2D_3$- and vehicle-treated mice (Table 1) indicating that $1,25(OH)_2D_3$ did not influence the phenotype of DCs present in the bone marrow. When analyzing DCs purified from thymus, a significant up-regulation of CD86 expression could be observed for the in vivo $1,25(OH)_2D_3$ treated NOD mice (Table 2). This brought the NOD DCs more towards a NOR phenotype.

Next, we generated DCs in vitro from bone marrow of vehicle- and $1,25(OH)_2D_3$-treated NOR and NOD mice. Therefore, bone marrow cells were cultured for 8 days in the presence of 20 ng/ml murine recombinant GM-CSF (Peprotech, Rocky Hill, NJ, USA) and 20 ng/ml murine recombinant IL-4 (Peprotech) for stimulating DC growth. An additional culture period of 2 days in the presence of 10 ng/ml murine recombinant IFN-γ (Peprotech) and 1000 ng/ml LPS (Sigma, St Louis, MO, USA) stimulated DC maturation. On day 10 of culture, cells were harvested and analyzed by FACS for their surface expression of MHC II, CD86, CD40 and CD54 within the $CD11c^+$ window. Surprisingly, only an up-regulation of MHC II was observed in NOR mice (from an MFI of 255 ± 142 for vehicle-treated mice to 373 ± 45 for $1,25(OH)_2D_3$-treated mice, $P < 0.05$). No changes in CD86, CD40 or CD54 expression levels were seen. These data indicate that in bone marrow no long term changes are induced by in vivo $1,25(OH)_2D_3$ treatment. However, when DCs are generated in vitro from bone marrow of vehicle- and $1,25(OH)_2D_3$-treated mice and additionally treated during the whole in vitro culture period with 10^{-8} M $1,25(OH)_2D_3$, important effects, paralleling our previous findings in human peripheral blood derived DCs [4], could be observed (Table 3). The expression of MHC II, CD86 and CD54 was significantly down-regulated to the same extend as in vehicle treated NOD mice.

Table 2
The effects of in vivo $1,25(OH)_2D_3$ treatment on the surface expression of MHC II, CD86, CD40 and CD54 on $CD11c^+$ DCs purified from thymus of NOR and NOD mice

	NOR		NOD	
	Vehicle	$1,25(OH)_2D_3$	Vehicle	$1,25(OH)_2D_3$
MHC II (MFI)[a]	94 ± 13	nd[b]	120 ± 33	115 ± 3
CD86 (MFI)	48 ± 5	nd	33 ± 5	42 ± 7*
CD40 (MFI)	901 ± 341	nd	626 ± 333	409 ± 167
CD54 (MFI)	284 ± 31	nd	335 ± 24	326 ± 32

Mice were vehicle-treated or $1,25(OH)_2D_3$-treated (5μg/kg three times weekly) from weaning to 10 weeks of age. Cells were harvested from thymus and $CD11c^+$ DCs were purified by MACS and analyzed by FACS for surface expression of MHC II, CD86, CD40 and CD54 within the $CD11c^+$ window. The results are expressed as mean ± S.D.

[a] Values for MHC II, CD86 and CD40, CD54 were expressed as mean fluorescence intensity (MFI).

[b] nd: not done.

* $P < 0.05$ compared to vehicle–treated NOD mice.

3. Early life treatment with vitamin D or $1,25(OH)_2D_3$ in NOD mice

Also in humans evidence exists suggesting a role for the vitamin D system in the pathogenesis of type 1 diabetes. Polymorphisms of the vitamin D receptor gene as well as geographical distribution have been associated with type 1 diabetes [11–13]. Dietary vitamin D supplementation during infancy is associated with a reduced risk of type 1 diabetes later in life [14–16]. In NOD mice, early life treatment (being from 3 to 70 days of age) with vitamin D or with $1,25(OH)_2D_3$ has direct protective effects on the insulin producing beta cells [17,18]. Although diabetes could not be prevented, a preservation of the beta cell mass in the islets could be observed, confirming earlier data showing that, besides effects on the immune system, vitamin D and $1,25(OH)_2D_3$ also have direct effects on the beta cell itself making them more resistant to autoimmune mediated destruction [19,20]. Besides the NOD mouse, the biobreeding (BB) rat is another animal model developing

Table 3
The effects of in vitro $1,25(OH)_2D_3$ treatment on the surface expression of MHC II, CD86, CD40 and CD54 on $CD11c^+$ DCs purified from vehicle- and $1,25(OH)_2D_3$-treated NOD mice

	Vehicle-treated NOD		$1,25(OH)_2D_3$-treated NOD	
	Control	$1,25(OH)_2D_3$	Control	$1,25(OH)_2D_3$
MHC II (MFI)[a]	204 ± 88	109 ± 40*	241 ± 93	102 ± 74*
CD86 (MFI)	313 ± 154	79 ± 34*	311 ± 198	45 ± 15*
CD40 (MFI)	573 ± 210	695 ± 348	476 ± 66	623 ± 520
CD54 (%)[b]	63 ± 19	48 ± 22*	69 ± 22	31 ± 10*

Mice were vehicle-treated or $1,25(OH)_2D_3$-treated (5 μg/kg three times weekly) from weaning to 10 weeks of age. Bone marrow cells were harvested from femurs and tibia and cultured for 8 days in the presence of GM-CSF + IL-4 and for 2 more days with IFN-γ + LPS, with ($1,25(OH)_2D_3$) or without (control) the addition of 10^{-8} M $1,25(OH)_2D_3$ during the whole in vitro culture period. On day 10, cells were analyzed by FACS for surface expression of MHC II, CD86, CD40 and CD54 within the $CD11c^+$ window.

[a] Values for MHC II, CD86 and CD40 were expressed as mean fluorescence intensity (MFI).

[b] Values for CD54 were expressed as % CD54 high positive cells.

* $P < 0.05$ vs. control-treated mice with the same in vivo treatment.

Table 4
The effects of early life treatment with vitamin D or $1,25(OH)_2D_3$ on the diabetes incidence in BB rats

	Diabetes incidence (% (numbers))	Day of onset (Mean ± S.D. (range))
Vehicle	20 (6/30)	114 ± 15 (91–133)
Vitamin D (1000 IU/d)	29 (8/28)	111 ± 20 (79–149)
$1,25(OH)_2D_3$ (0.2 μg/kg per day)	29 (9/31)	104 ± 24 (72–150)
$1,25(OH)_2D_3$ (1 μg/kg per 2 days)	39 (13/33)	101 ± 12 (86–126)

Rats were treated intraperitoneally from 3 to 50 days of age with vitamin D or one of both $1,25(OH)_2D_3$ regimens. Vehicle-treated rats served as controls. Animals were followed up for diabetes incidence until 30 weeks of age.

spontaneous diabetes. Also in this model the effects of early life treatment with vitamin D and $1,25(OH)_2D_3$ on diabetes development was investigated.

Therefore BB rats were treated intraperitoneally with vitamin D (1000 IU per day) or with $1,25(OH)_2D_3$ (1 μg/kg every other day or 0.2 μg/kg per day) from 3 to 50 days of age, again corresponding to early childhood in humans, and followed up for diabetes incidence until 30 weeks of age. Vehicle treated rats served as control. Results are summarized in Table 4. Early life treatment with vitamin D or with $1,25(OH)_2D_3$ could not significantly prevent diabetes in BB rats. In addition, by none of the treatment regimens the day of diabetes onset was significantly altered. Whether early life treatment with vitamin D or $1,25(OH)_2D_3$ could preserve the beta cell mass in the islets of BB rats is still under investigation.

4. Vitamin D deficiency in early life accelerates diabetes in NOD mice

Besides the studies showing that vitamin D supplementations early in life reduce the risk for type 1 diabetes [14–16], a Finish study demonstrates that overt rickets in the first year of life induces a 3-fold increase in the prevalence of type 1 diabetes [21]. This effect of vitamin D deficiency in children is much greater than the effects of early life vitamin D supplementations, suggesting that mainly the vitamin D deficient status triggers autoimmunity while for preventing autoimmunity high doses of the active $1,25(OH)_2D_3$ are needed. Based on this knowledge, we investigated the effects of vitamin D deficiency in utero and in early life on the prevalence of type 1 diabetes in NOD mice.

With the purpose of inducing vitamin D deficiency, male and female NOD mice were kept from 3 weeks onward in UV-free surroundings and fed with a vitamin D depleted diet (Harlan Teklad Test Diets, Madison, WI). These mice were used for breeding and the experiments were carried out with their offspring who were also kept in UV-free surroundings and fed the vitamin D deficient chow until 100 days of age where after they received normal vitamin D supplemented chow (2000 IU/kg, Harlan Teklad). Age and gender-matched NOD mice kept in the same room but fed with normal vitamin D supplemented chow were used as controls. Mice were followed up for diabetes incidence until 250 days of age, after all being switched to a normal diet at 100 days. In NOD mice that were vitamin D deficient in early life, diabetes incidence was nearly doubled compared to gender matched control animals (Figure 1). While only 6/40 control male NOD mice had diabetes at 250 days of age, 12/35 vitamin D deficient male NOD mice developed diabetes by that time ($P < 0.05$). In the female population, 13/29 control female NOD mice versus 22/33 vitamin D deficient female NOD mice ($P < 0.01$) became diabetic by 250 days of age. Not only was the diabetes incidence increased in the vitamin D deficient NOD mice, also the onset of the disease occurred much earlier. While the mean day of diabetes onset was 153 ± 20 days for male and 127 ± 22 days for female control NOD mice, in vitamin D deficient male and female NOD mice diabetes started at 113 ± 19 ($P < 0.0005$) and 108 ± 23 ($P < 0.0005$) days of age, respectively. These data demonstrate that a transient vitamin

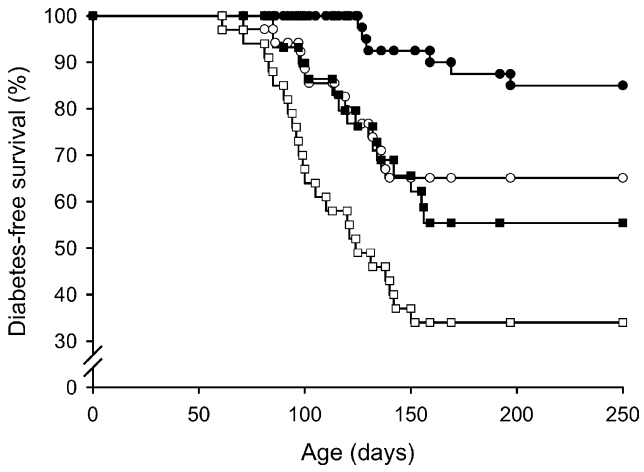

Fig. 1. Diabetes free survival of vitamin D deficient and control NOD mice. Vitamin D deficiency was induced in breeding couples by keeping them in UV-free surroundings and on a vitamin D depleted diet. Their offspring were also kept in UV-free surroundings and on a vitamin D depleted diet until 100 days of age, afterwards they received normal vitamin D supplemented chow. This second generation was used to determine the diabetes free survival. Age and gender-matched NOD mice kept in UV-free surroundings but on normal vitamin D supplemented chow were used as controls. Mice were followed up for diabetes incidence until 250 days of age. Results are expressed as percentage diabetes free survival in normal (●) and rachitic (○) male NOD mice, and normal (■) and rachitic (□) female NOD mice.

D deficiency in utero and in early life leads to a more aggressive occurrence of type 1 diabetes in NOD mice with an earlier onset and a lower disease free survival. The effects of early life vitamin D deficiency on the different parts of the immune system are being investigated in more detail, in order to understand how diabetes is aggravated in NOD mice. Extrapolating these data to the human situation, strictly controlling vitamin D status during pregnancy and in early life could be an easy and especially safe way to reduce the incidence of type 1 diabetes in at risk populations.

5. Conclusions

We conclude that prevention of diabetes by vitamin D metabolites in NOD mice can only be achieved by long term treatment with $1,25(OH)_2D_3$. However, vitamin D deficiency in early life in these high risk mice leads to a dramatic increase in diabetes. Avoiding vitamin D deficiency in high risk individuals could be a safe and easy way to reduce diabetes incidence in humans.

References

[1] C.M. Veldman, M.T. Cantorna, H.F. DeLuca, Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system, Arch. Biochem. Biophys. 374 (2) (2000) 334–338.

[2] G. Penna, L. Adorini, 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation, J. Immunol. 164 (5) (2000) 2405–2411.

[3] L. Piemonti, P. Monti, M. Sironi, P. Fraticelli, B.E. Leone, E. Dal Cin, P. Allavena, C. Di, V, Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells, J. Immunol. 164 (9) (2000) 4443–4451.

[4] A.G. van Halteren, E. van Etten, E.C. de Jong, R. Bouillon, B.O. Roep, C. Mathieu, Redirection of human autoreactive T-cells upon interaction with dendritic cells modulated by TX527, an analog of 1,25 dihydroxyvitamin D(3), Diabetes 51 (7) (2002) 2119–2125.

[5] C. Mathieu, L. Adorini, The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents, Trends Mol. Med. 8 (4) (2002) 174–179.

[6] C. Mathieu, M. Waer, J. Laureys, O. Rutgeerts, R. Bouillon, Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3, Diabetologia 37 (6) (1994) 552–558.

[7] C. Mathieu, J. Laureys, H. Sobis, M. Vandeputte, M. Waer, R. Bouillon, 1,25-Dihydroxyvitamin D3 prevents insulitis in NOD mice, Diabetes 41 (11) (1992) 1491–1495.

[8] M. Feili-Hariri, P.A. Morel, Phenotypic and functional characteristics of BM-derived DC from NOD and non-diabetes-prone strains, Clin. Immunol. 98 (1) (2001) 133–142.

[9] J. Strid, L. Lopes, J. Marcinkiewicz, L. Petrovska, B. Nowak, B.M. Chain, T. Lund, A defect in bone marrow derived dendritic cell maturation in the non-obese diabetic mouse, Clin. Exp. Immunol. 123 (3) (2001) 375–381.

[10] S. Boudaly, J. Morin, R. Berthier, P. Marche, C. Boitard, Altered dendritic cells (DC) might be responsible for regulatory T cell imbalance and autoimmunity in nonobese diabetic (NOD) mice, Eur. Cytokine Netw. 13 (1) (2002) 29–37.

[11] B.P. Koeleman, G. Valdigem, P. Eerligh, M.J. Giphart, B.O. Roep, Seasonality of birth in patients with type 1 diabetes, Lancet 359 (9313) (2002) 1246–1247.

[12] M.A. Pani, M. Knapp, H. Donner, J. Braun, M.P. Baur, K.H. Usadel, K. Badenhoop, Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans, Diabetes 49 (3) (2000) 504–507.

[13] A. Green, E.A. Gale, C.C. Patterson, Incidence of childhood-onset insulin-dependent diabetes mellitus: the EURODIAB ACE study, Lancet 339 (8798) (1992) 905–909.

[14] Vitamin D supplement in early childhood and risk for type I (insulin-dependent) diabetes mellitus, The EURODIAB Substudy 2 Study Group, Diabetologia 42 (1) (1999) 51–54.

[15] L.C. Stene, J. Ulriksen, P. Magnus, G. Joner, Use of cod liver oil during pregnancy associated with lower risk of type I diabetes in the offspring, Diabetologia 43 (9) (2000) 1093–1098.

[16] S. Harris, Can vitamin D supplementation in infancy prevent type 1 diabetes? Nutr. Rev. 60 (4) (2002) 118–121.

[17] C. Mathieu, E. van Etten, C. Gysemans, B. Decallonne, R. Bouillon, Seasonality of birth in patients with type 1 diabetes, Lancet 359 (9313) (2002) 1248.

[18] E. van Etten, B. Decallonne, C. Mathieu, 1,25-dihydroxycholecalciferol: endocrinology meets the immune system, Proc. Nutr. Soc. 61 (3) (2002) 375–380.

[19] R. Riachy, B. Vandewalle, S. Belaich, J. Kerr-Conte, V. Gmyr, F. Zerimech, M. d'Herbomez, J. Lefebvre, F. Pattou, Beneficial effect of 1,25 dihydroxyvitamin D3 on cytokine-treated human pancreatic islets, J. Endocrinol. 169 (1) (2001) 161–168.

[20] H.J. Hahn, B. Kuttler, C. Mathieu, R. Bouillon, 1,25-Dihydroxyvitamin D3 reduces MHC antigen expression on pancreatic beta-cells in vitro, Transplant. Proc. 29 (4) (1997) 2156–2157.

[21] E. Hypponen, E. Laara, A. Reunanen, M.R. Jarvelin, S.M. Virtanen, Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study, Lancet 358 (9292) (2001) 1500–1503.