Tolerogenic Dendritic Cells Induced by Vitamin D Receptor Ligands Enhance Regulatory T Cells Inhibiting Autoimmune Diabetes

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ABSTRACT: Dendritic cells (DCs) not only induce but also modulate T cell activation. 1,25-Dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] induces DCs with a tolerogenic phenotype, characterized by decreased expression of CD40, CD80, and CD86 co-stimulatory molecules, low IL-12, and enhanced IL-10 secretion. We have found that a short treatment with 1,25-$(OH)_2D_3$ induces tolerance to fully mismatched mouse islet allografts, and that this tolerance is stable to challenge with donor-type spleen cells and allows acceptance of donor-type vascularized heart grafts. This effect is enhanced by co-administration of mycophenolate mofetil (MMF), a selective inhibitor of T and B cell proliferation, that also has effects similar to 1,25-$(OH)_2D_3$ on DCs. Graft acceptance is associated with impaired development of type 1 $CD4^+$ and $CD8^+$ cells and an increased percentage of $CD4^+CD25^+$ regulatory cells expressing CD152 in the spleen and in the draining lymph node. Transfer of $CD4^+CD25^+$ cells from tolerant mice protects 100% of the syngeneic recipients from islet allograft rejection. $CD4^+CD25^+$ cells that are able to inhibit the T cell response to a pancreatic autoantigen and to significantly delay disease transfer by pathogenic $CD4^+CD25^-$ cells are also induced by treatment of adult nonobese diabetic (NOD) mice with a selected vitamin D receptor (VDR) ligand. This treatment arrests progression of insulitis and Th1 cell infiltration, and inhibits diabetes development at non-hypercalcemic doses. The enhancement of $CD4^+CD25^+$ regulatory T cells able to mediate transplantation tolerance and to arrest type 1 diabetes development by a short oral treatment with small organic compounds that induce tolerogenic DCs, like VDR ligands, suggests possible clinical applications of this approach.

KEYWORDS: dendritic cells; vitamin D receptor; type 1 diabetes

MAJOR TARGETS OF VDR LIGANDS IN THE IMMUNE SYSTEM: ANTIGEN-PRESENTING CELLS AND T CELLS

The active form of vitamin D, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], is a secosteroid hormone that binds to the vitamin D receptor (VDR), a member of the superfamily of nuclear receptors for steroid hormones, thyroid hormone, and

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retinoic acid. VDR ligands regulate calcium and bone metabolism, control cell proliferation and differentiation, and exert immunoregulatory activities. An important property of VDR ligands is their capacity to modulate both antigen-presenting cells (APCs) and T cells. Thus, these agents can target T cells both directly and indirectly, via modulation of APC function. VDR ligands inhibit the differentiation and maturation of DCs,[1] a critical APC in the induction of T cell-mediated immune responses. These studies, performed either on monocyte-derived DCs from human peripheral blood or on bone-marrow-derived mouse DCs, have consistenly shown that *in vitro* treatment of DCs with 1,25-$(OH)_2D_3$ and its analogs leads to down-regulated expression of the co-stimulatory molecules CD40, CD80, and CD86, and to decreased IL-12 and enhanced IL-10 production, resulting in decreased T cell activation. The abrogation of IL-12 production and the strongly enhanced production of IL-10 highlight the important functional effects of 1,25-$(OH)_2D_3$ and its analogues on DCs and are, at least in part, responsible for the induction of DCs with tolerogenic properties.[2]

These effects are not limited to *in vitro* activity: VDR ligands can also induce DCs with tolerogenic properties *in vivo*, as demonstrated in models of allograft rejection by oral administration directly to the recipient[3] or by adoptive transfer of *in vitro*-treated DCs.[4] Tolerogenic DCs induced by 1,25-$(OH)_2D_3$ can, in turn, induce $CD4^+CD25^+$ regulatory T cells that are able to mediate transplantation tolerance.[3] This is a novel aspect of the multiple effects of VDR ligands on T cells. In addition, a combination of 1,25-$(OH)_2D_3$ and dexamethasone has been shown to induce human and mouse naive $CD4^+$ T cells to differentiate *in vitro* into regulatory T cells.[5] These agents induced the development of IL-10-producing T cells also in the absence of APCs, with IL-10 acting as a positive autocrine factor.[5]

IMMUNOMODULATORY EFFECTS OF 1,25-$(OH)_2D_3$ AND ITS ANALOGUES IN AUTOIMMUNE DIABETES

The nonobese diabetic (NOD) mouse spontaneously develops type 1 diabetes, a Th1-mediated chronic progressive autoimmune disease, by targeting pancreatic β cells with a pathogenesis similar to the human disease.[6] Agents such as VDR ligands, which are able to inhibit *in vivo* IL-12 production and Th1 development,[7] and to enhance $CD4^+CD25^+$ regulatory T cells,[3] may therefore be beneficial in the treatment of type 1 diabetes. 1,25-$(OH)_2D_3$ itself reduces the incidence of insulitis and prevents type 1 diabetes development, but only when administered to NOD mice starting from three weeks of age, before the onset of insulitis.[8]

In contrast, we have recently identified the VDR ligand 1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor vitamin D_3 (Ro 26-2198) that is able, as a monotherapy, to treat the ongoing type 1 diabetes in the adult NOD mouse, effectively blocking the disease course.[9] This property is likely due, at least in part, to the increased metabolic stability of this analogue against the inactivating C-24 and C-26 hydroxylations, and the C-3 epimerization, resulting in a 100-fold more potent immunosuppressive activity compared to 1,25-$(OH)_2D_3$. A short treatment with non-hypercalcemic doses of Ro 26-2198 inhibits IL-12 production and pancreatic infiltration of Th1 cells while increasing the frequency of $CD4^+CD25^+$ regulatory T cells in pancreatic lymph nodes, arresting the immunological progression and preventing the clinical onset of type 1 diabetes in the NOD mouse.[9] The frequency of

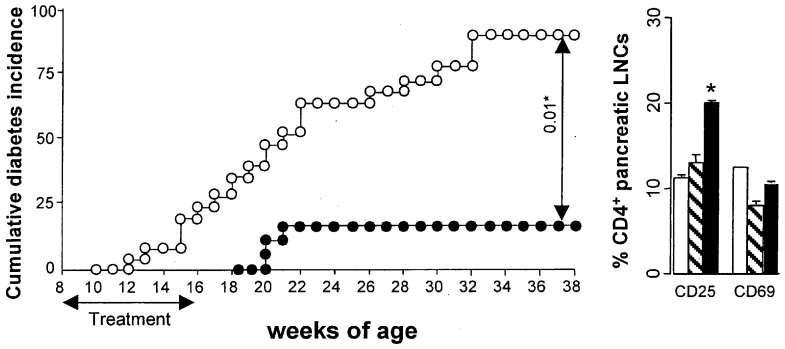

FIGURE 1. Ro 26-2198 administration to 8-week-old NOD mice inhibits the development of type 1 diabetes. NOD mice were treated five times each week with vehicle (*open circles*, $n = 16$) or with 0.03 μg/kg Ro 26-2198 p.o. (*filled circles*, $n = 12$) from 8 to 16 weeks of age. The development of diabetes was monitored twice weekly by measurement of blood glucose levels. Ro 26-2198 treatment enhances the frequency of $CD4^+CD25^+$ cells. Positively selected pancreatic lymph node $CD4^+$ T cells were stained with monoclonal antibodies specific for CD25 or CD69 molecules and analyzed by flow cytometry. Bars represent the percentage of lymph node $CD4^+$ T cells expressing the indicated surface molecules from untreated 8-week-old (*open bars*) or 20-week-old mice treated five times each week, from 8 to 16 weeks of age, with vehicle (*striped bars*) or with 0.03 μg/kg Ro 26-2198 (*filled bars*). Each value represents a mean ± SE of three separate experiments. The *P* values were calculated via the Mann-Whitney U test ($^* P < 0.05$ vs. 8 week-old NOD mice).

$CD4^+CD25^+$ cells in the pancreatic lymph nodes of Ro 26-2198-treated NOD mice was twofold higher compared to untreated 8-week-old and to age-matched vehicle-treated controls. These cells were anergic, as demonstrated by their impaired capacity to proliferate and secrete IFN-γ in response to TCR ligation, inhibited the T cell response to the pancreatic autoantigen IA-2, and delayed disease transfer by pathogenic $CD4^+CD25^-$ cells.[9]

Immature DCs have been shown to induce $CD4^+$ cells with regulatory properties, and arrest of DCs at the immature stage induced by Ro 26-2198 treatment could account for the enhanced frequency of $CD4^+CD25^+$ cells. $CD4^+CD25^+$ regulatory T cells appear to play an important role in controlling the progression of type 1 diabetes in NOD mice, because a low level of $CD4^+CD25^+$ T cells correlates with exacerbation and acceleration of the disease. It is likely that this cell population is more relevant than Th2 cells in disease control, although both could contribute to protection. Indeed, $1,25\text{-}(OH)_2D_3$ can induce regulatory cells with disease-suppressive activity in the NOD mouse,[8] and Ro 26-2198 could contribute to the deviation of pancreas-infiltrating cells to the Th2 phenotype.[9]

Polymorphysms of the vitamin D receptor gene have been associated with type 1 diabetes in different populations, and epidemiological studies have suggested a correlation with calcitriol levels. This is further supported by a large population-based case-control study showing that the intake of vitamin D contributes to a significantly decreased risk of type 1 diabetes development.[10] The observation that ongoing type 1 diabetes in the adult NOD mouse can be arrested by a relatively short course of

treatment with a VDR ligand suggests that a similar treatment may also inhibit disease progression in prediabetic or newly diagnosed type 1 diabetes patients.

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