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1α,25-Dihydroxyvitamin D_3 restores thymocyte apoptosis sensitivity in non-obese diabetic (NOD) mice through dendritic cells

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Abstract

Aims/hypothesis: Resistance of NOD thymocytes to apoptosis-inducing signals is restored by 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), a therapy preventing diabetes in NOD mice. We studied whether modulation of thymocyte apoptosis is due to direct effects on thymic T lymphocytes or indirect effects via thymic dendritic cells, since both cell types constitute known targets for 1α,25$(OH)_2D_3$.

Methods and results: Female NOD mice were treated with 1α,25$(OH)_2D_3$ (5 μg/kg/2d) from 21 to 70 days. Vehicle-treated NOD and NOR mice served as controls. Analysis of thymic T lymphocytes from 1α,25$(OH)_2D_3$-treated mice revealed a decrease in number of apoptosis-resistant $CD4^+CD8^+$ and $CD4^+CD8^-HSA^{high}$ T lymphocyte subsets, higher pro-apoptotic IL-2 and FasL, and lower anti-apoptotic Bclx-L mRNA expression levels. Thymic dendritic cells from 1α,25$(OH)_2D_3$-treated NOD mice had increased $CD8\alpha^+FasL^+$ and $CD80^+/86^+$ expression compared to control NOD mice. In a syngeneic co-culture system of thymocytes and thymic dendritic cells, apoptosis levels were 20% higher only in co-cultures where both T cell- and dendritic cell-compartments originated from 1α,25$(OH)_2D_3$-treated mice. Activation-induced cell death-sensitivity in peripheral T lymphocytes was comparable to levels present in NOR mice, confirming better thymic selection in 1α,25$(OH)_2D_3$-treated mice.

Conclusion/interpretation: We conclude that 1α,25$(OH)_2D_3$ needs both thymic T cell- and dendritic cell-compartments to exert its apoptosis-restorative effects in NOD thymocytes.

Keywords: Type 1 diabetes; Apoptosis; NOD mice; Thymus; Vitamin D

1. Introduction

Type 1 diabetes is an organ-specific T lymphocyte-mediated autoimmune disease, characterised by destruction of insulin producing pancreatic β cells with subsequent development of hyperglycaemia and insulin dependence. Since exogenous insulin therapy is not able to completely avoid long-term complications and subsequent enormous health care costs, an etiologic more than a purely symptomatic treatment is urgently needed, aiming at prevention of diabetes. Prevention, however, supposes insight in the early pathogenic mechanisms,

Abbreviations: NOD, non-obese diabetic; NOR, non-obese diabetes-resistant; 1α,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; DC, dendritic cell; APC, antigen-presenting cell; mAb, monoclonal antibody; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated FITC-dUTP nick end labelling reaction; AICD, activation-induced cell death.

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before disease becomes overt. Previous studies in pre-diabetic NOD mice, a mouse model for human type 1 diabetes, demonstrated several defects in the immune system, both in the T cell and antigen-presenting cell (APC) compartments [1]. Immunomodulation at young age, aiming at re-setting one or several immune dysregulations, thus seems a logical approach in the prevention of autoimmune diabetes. Life long treatment with high doses of 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$), the activated form of vitamin D, prevents diabetes in NOD mice [2]. Further research on its mechanism of action in primary prevention revealed that $1\alpha,25(OH)_2D_3$ induced a restoration of T lymphocyte sensitivity to in vivo apoptosis-inducing signals (dexamethasone, cyclophosphamide), especially in the thymus [3,4]. No general immune suppression was observed.

Abnormal T cell selection, which has been reported in NOD mice [5,6], can be due to aberrations in the T lymphocytes themselves or in the thymic APC compartment, responsible for the presentation of (auto)antigen to thymic T lymphocytes. Dendritic cells (DCs) represent crucial APCs and several DC abnormalities have been described in NOD mice [7–12]. Moreover, DCs constitute important target cells for the action of $1\alpha,25(OH)_2D_3$ [13]. The aim of this work was to study whether modulation of thymocyte apoptosis sensitivity by $1\alpha,25(OH)_2D_3$ is due to direct effects on T lymphocytes or indirect effects on thymic DCs. The data presented here demonstrate that direct effects of $1\alpha,25(OH)_2D_3$ on T lymphocytes combined with indirect effects on DCs are indispensable for final modulation of central T cell apoptosis sensitivity.

2. Materials and methods

2.1. Animals

NOD mice, originally obtained from Professor Wu (Bejing, China) were housed and inbred in our animal facility since 1989. Housing occurred under semi-barrier conditions and animals were fed sterile chow and water ad libitum [14]. The principles of laboratory animal care were followed (NIH publication no. 85-23, revised 1985) and all experiments were approved by the local ethical committee for animal experiments of the Catholic University of Leuven. Insulitis develops from 4 weeks onward, reaching an incidence of 80% at 70 days of age and >95% at 200 days of age. At the time of the experiments, the cumulative incidence of spontaneous diabetes in our colony ranged from 55 to 76% in females and from 10 to 52% in males of 200 days of age. Non-diabetic female NOD mice aged 8–12 weeks were used. Age-matched female NOR/Ltj mice (Jackson Laboratories, Bar Harbor, ME) were used as an MHC-matched diabetes-resistant control strain for NOD mice [15].

2.2. $1\alpha,25(OH)_2D_3$ treatment regimen

$1\alpha,25(OH)_2D_3$, kindly provided by JP Van de Velde (Solvay, Weesp, The Netherlands), was administered intraperitoneally at a dose of 5 μg/kg every other day from the age of 3 weeks until sacrifice. The control group was treated with arachis oil (vehicle).

2.3. Ex vivo thymic DC and T lymphocyte isolation

Thymuses were isolated after the mice were ether-anesthetised and bled. CD11c (the integrin-α_x chain) was considered as marker for mature thymic DCs. Within the thymus, only DCs express CD11c. DCs were isolated from thymuses with CD11c-magnetically labelled beads (Miltenyi Biotec, Bergisch Gladbach, Germany) using magnetic cell sorting (MACS) technology. Briefly, 2–6 thymuses were pooled per experimental group and per experiment. A single cell suspension was prepared and cells were counted. The cell suspension was incubated on ice with the CD11c-magnetically labelled beads (10 μl beads + 40 μl sterile PBS supplemented with 0.5% BSA and 2 mM EDTA (further referred to as PBS^+)/10×10^6 cells) during 20 min. Cells were then washed and resuspended in a constant volume of 500 μl PBS^+. Positive selection of CD11c-magnetically labelled cells was performed on LS columns (Miltenyi Biotec) following the manufacturer's protocol. Purity was determined by FITC-CD11c mAb (clone HL3, BD Pharmingen, San Diego, CA) staining and subsequent flow cytometric analysis (FACScan, Becton Dickinson, Mountain View, CA) using Cell Quest software. By this procedure $CD11c^+$ thymic DCs were enriched 30-fold (from 0.5 ± 0.3% in total thymus cell population to 15 ± 3% after MACS CD11c-positive selection). The thymic CD11c-negative fraction obtained after MACS, containing >98% T lymphocytes, was considered as T lymphocyte fraction.

2.4. Surface phenotyping of dendritic cells and T lymphocytes

CD11c was used as marker for DCs. For thymic DCs, representing only 0.5% of the total thymic cell population, a two-step purification was performed in order to enable adequate ex vivo DC phenotyping of a sufficient number of DCs. First, CD11c-positive selection was performed with MACS as described above, resulting in ±15% $CD11c^+$ cells. Second, all samples were double-stained with CD11c mAb (FITC or PE, clone HL3) and one of the surface markers of interest: anti-mouse PE-CD11b (clone M1/70), FITC-CD40 (clone HM 40-3), PE-CD54 (clone 3E2),