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NOD bone marrow-derived dendritic cells are modulated by analogs of 1,25-dihydroxyvitamin D_3 ☆

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Abstract

The immune effects of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) are mainly mediated through dendritic cells (DCs). In vitro, $1,25(OH)_2D_3$ treatment renders murine bone marrow (BM)-derived DCs more tolerogenic, indirectly altering behavior and fate of T lymphocytes. In vivo, treatment with $1,25(OH)_2D_3$ or its analogs prevents diabetes in NOD mice. The aim of this study was to investigate the effects of the $1,25(OH)_2D_3$-analog TX527 on the expression of antigen-presenting and costimulatory/migratory molecules on BM-derived DCs from NOD mice. After culture with 20 ng/ml GM-CSF + 20 ng/ml IL-4 (8 days) followed by 1000 ng/ml LPS + 100 U/ml IFN-γ (2 days), with or without 10^{-8} M TX527, cells were counted and analyzed by FACS for MHC II, CD86, CD40 and CD54 expression within the $CD11c^+$ DC population. Upon TX527 treatment, cell recovery was significantly reduced whereas the $CD11c^+$ DC fraction remained constant. On $CD11c^+$ DCs, MHC II, CD86 and CD54 were significantly down-regulated and CD40 was twofold upregulated. Globally, BM-derived DCs from NOD mice become more tolerogenic upon TX527 treatment, confirming the effects of $1,25(OH)_2D_3$ on murine DCs and possibly explaining the protective effects of $1,25(OH)_2D_3$ and its analogs from diabetes in NOD mice.
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1. Introduction

The effects of the active form of Vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), on the immune system are mainly mediated through its actions on antigen-presenting cells of which the dendritic cells (DCs) are the most potent population. In vitro treatment of human peripheral blood-derived DCs or murine bone marrow-derived DCs with $1,25(OH)_2D_3$ results in the inhibition of antigen-presentation and costimulatory molecules and the down-regulation of IL-12 production and secretion [1–6]. As a result, proliferation of T lymphocytes is inhibited and a more regulatory panel of cytokines is produced. Redirection of committed human autoreactive T cell clones is even achieved by exposure to $1,25(OH)_2D_3$-modulated DCs without the additional presence of $1,25(OH)_2D_3$ itself [3]. In vivo treatment of spontaneously diabetic non-obese diabetic (NOD) mice with $1,25(OH)_2D_3$ prevents diabetes and insulitis and even reduces the recurrence of autoimmunity after transplantation of syngeneic as well as xenogeneic islets [7–10]. A major problem when using $1,25(OH)_2D_3$ in vivo are its effects on calcium and bone metabolism due to which diabetes protection can only be achieved with doses near the toxic range. Structural analogs of $1,25(OH)_2D_3$ have been made with a dissociation between calcemic and immune effects [11,12], some of which are able to reduce the incidence of insulitis and diabetes in NOD mice without any effects on calcium and bone metabolism [13–15].

Based on the facts that $1,25(OH)_2D_3$-mediated immune effects occur mainly at the level of antigen-presenting cells such as DCs, and that in vivo treatment with $1,25(OH)_2D_3$ and its analogs protect NOD mice from diabetes, we investigated the effects of a structural analog of $1,25(OH)_2D_3$, TX527 (19-nor-14,20-bisepi-23-yne-$1,25(OH)_2D_3$), on bone marrow-derived DCs from NOD mice.

2. Materials and methods

2.1. Structural analog of $1,25(OH)_2D_3$: TX527

The Vitamin D_3 analog TX527 (19-nor-14,20-bisepi-23-yne-$1,25(OH)_2D_3$; [16]) was first synthesized by P. Declercq and M. Vandewalle (Laboratory of Organic Chemistry, University of Gent, Gent, Belgium) and further provided by J.C.

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Pascal and N. Adje (Théramex SA, Monaco). The analog was dissolved in ethanol and further in culture medium diluted to the concentration needed.

2.2. Mice

Non-obese diabetic mice were kindly provided by Professor C.Y. Wu (Bejing, China) and have been inbred in our animal facility since 1989. Housing of NOD was under semi-barrier conditions. All experiments were performed using female NOD mice between 8 and 14 weeks of age. All animal breeding and experimental protocols were approved by the ethical committee of the Katholieke Universiteit Leuven (project no. P98037).

2.3. In vitro generation of DCs from bone marrow

Bone marrow-derived DCs were generated using a modification of a previously described protocol [17]. Briefly, bone marrow cells, harvested from femurs and tibia, were counted and resuspended in RPMI 1640 medium (GIBCO, Paisley, Scotland) supplemented with Glutamax-I (Gibco), 25 mM HEPES (Gibco), 5 μg/ml geneticin (Life Technologies, Rockville, MD, USA), 10% heat-inactivated fetal calf serum (FCS, Gibco) and 5×10^{-5} M β-mercapto-ethanol (UCB, Brussels, Belgium). Cells were plated in six-well plates at a concentration of 1×10^6 ml^{-1} and incubated at 37 °C in humidified air with 5% CO_2. For the first 8 days of culture, medium was supplemented with 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. Medium was refreshed on days 3 and 6. 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 the 10th day of culture, non-adherent cells were harvested and counted. This in vitro DC generation was performed with or without 10^{-8} M TX527, added every time the medium was refreshed.

2.4. Surface marker expression

After 10 days of culture, non-adherent cells were analyzed for their surface marker expression by fluorescence-activated cell sorting (FACS). Cells were double-stained with antibodies against CD11c and one of the following surface markers: MHC II (IA^k), CD86, CD40 and CD54 (Becton Dickinson, San Jose, CA, USA). After extensive washing and fixation in 2% paraformaldehyde, cells were analyzed on a FACSort (Becton Dickinson).

2.5. Statistical analysis

For comparing the results, Student's *t*-tests were used. Results were statistically different when $P < 0.05$ and were expressed as mean ± standard deviation.

Table 1
TX527 treatment during the in vitro generation of bone marrow-derived DCs from NOD mice changes surface marker expression on $CD11c^+$ cells

	Control	TX527
MHC II (MFI)[a]	204 ± 88	98 ± 25*
CD86 (MFI)	313 ± 154	52 ± 10**
CD40 (MFI)	573 ± 210	1342 ± 328***
CD54 (%)[b]	63 ± 19	13 ± 7***

Bone marrow cells from female NOD mice were cultured for 8 days in the presence of GM−CSF+IL-4 and for 2 more days with IFN−γ+LPS, with or without the addition of 10^{-8} M TX527 during the whole culture period. On day 10, cells were analyzed by FACS for surface expression of MHC II, CD86, CD40 and CD54 within the $CD11c^+$ window. Statistically significant differences are expressed as: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to control conditions.

[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.

3. Results

3.1. Cell counts

Treatment with TX527 during the generation of DCs from the bone marrow of NOD mice, resulted in an major decrease in cell yield after culture. Whereas on day 10 almost one quarter of the initial amount of cells could be harvested in the control cultures (23 ± 12%), TX527 treatment reduced this yield to 2 ± 1% ($P < 0.001$). However, the fraction of $CD11c^+$ cells within the surviving populations, being a measure for the DC population, was not changed by TX527 treatment (21 ± 15% for control DCs and 36 ± 14% for TX527-treated DCs, NS).

3.2. Surface marker expression

Next we analyzed the expression of markers for antigen-presentation (MHC II), costimulation (CD86 and CD40) and adhesion (CD54) on the surface of $CD11c^+$ cells (Table 1). Upon TX527-treatment during DC generation, the expression of MHC II and CD86 was significantly decreased. In addition, the percentage of $CD11c^+$ cells that were highly positive for CD54 was significantly down-regulated. In contrast, the expression of CD40 was increased more than twofold after TX527 treatment.

4. Discussion

Our data demonstrate that in vitro treatment with TX527 did not affect the DC-generating capacity of NOD bone marrow cells but only decreased the total survival after culture. However not different in amount, the DCs generated in the presence of TX527 showed a different phenotype than those generated under control conditions. Surface expression of MHC II, CD86 and CD54 was reduced, pointing towards the

TX527-induced generation of DCs with a more tolerogenic phenotype. One interesting finding was the dramatic upregulation of CD40 expression by TX527. This is in contrast to the data on human DCs or murine bone marrow-derived DCs generated in the presence of $1,25(OH)_2D_3$ itself. The impact of the CD40 rise on the tolerogenic capacity of the DCs will have to be evaluated.

Globally, our data confirm the findings that $1,25(OH)_2D_3$ itself decreases antigen-presenting and costimulatory molecules on human peripheral blood-derived DCs [3–6] and murine bone marrow-derived DCs [18,2]. Moreover, the present data also are a confirmation of an earlier publication stating that the addition of TX527 altered in vitro differentiation and maturation of human peripheral blood-derived DCs [3]. Besides altered expression of surface molecules involved in antigen-presentation and costimulation, also morphology and IL-12 production of those human DCs was affected. Together, those TX527-induced changes created a DC with the potential to alter the response pattern of committed auto-reactive human T cell clones. Without the additional presence of TX527, TX527-modulated-DCs inhibited T-cell proliferation and blocked IFN-gamma, IL-10, but not IL-13 production. In conclusion, TX527 altered the phenotype of bone marrow-derived DCs from NOD mice, resulting in a cell with tolerogenic potential.

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