

Regulation of Cytokine Production in Human Peripheral Blood Mononuclear Cells and Allergen-Specific Th Cell Clones by $1\alpha,25$ -Dihydroxyvitamin D_3

Xiaohui Rausch-Fan^a Fritz Leutmezer^a Martin Willheim^a Andreas Spittler^b
Barbara Bohle^a Christof Ebner^a Erika Jensen-Jarolim^a
George Boltz-Nitulescu^a

^aInstitute of Pathophysiology, and ^bSurgical Research Laboratory, University of Vienna, Vienna, Austria

Key Words

Calcitriol · Vitamin D_3 · Cytokines · Lymphocytes · Th clones

Abstract

Background: The steroid hormone $1\alpha,25$ -dihydroxyvitamin D_3 (calcitriol), in addition to its crucial role in calcium homeostasis, exerts several effects on the immune system by regulating cell proliferation, differentiation, and maturation. These effects may be exerted through the control of protooncogenes and the regulation of cytokine production. **Methods:** The influence of calcitriol on cytokines secretion by human peripheral blood mononuclear cells (PBMC) isolated from healthy donors, and by allergen-specific T helper (Th) cell clones was studied. PBMC were cultured for 48 h with phorbol myristate acetate (PMA) and ionomycin in the presence or absence of calcitriol. Human Th cell clones were stimulated with either Bet v 1 allergen or anti-CD3 antibodies and PMA. Cytokines were measured in the supernatants by ELISA, and at single-cell level by FACS. **Results:** Calcitriol significantly inhibited the production of IL-2, IFN- γ and IL-12 by PBMC, as well as the percentage of CD4⁺ T cells contain-

ing intracytoplasmic IL-2 and IFN- γ . Interestingly, calcitriol-treated PBMC induced the production of IL-10 and IL-5, but not of IL-4. The effect of calcitriol was maximal at 10^{-7} to 10^{-9} and noneffective at 10^{-11} M. Calcitriol diminished the secretion of IL-1, TNF- α , and MG-CSF in PBMC. Furthermore, calcitriol also decreased the secretion of IL-2 and IFN- γ by Th1 clones, and of IL-4 by Th2 clones. **Conclusions:** Our data strongly support the notion that calcitriol modulates the production of cytokines in a time- and concentration-dependent manner, and suggest that nonhypercalcemic derivatives of $1\alpha,25$ -dihydroxyvitamin D_3 may be used for new immunosuppressive therapies.

Copyright © 2002 S. Karger AG, Basel

Introduction

Cytokines are soluble polypeptide cell products, have multiple sources, multiple targets and multiple functions. Based on the repertoire of cytokine production, the CD4⁺ lymphocytes are subdivided into T helper type 1 (Th1) and Th2 subsets [1]. The Th1 cells are defined by their ability to secrete the inflammatory cytokines IL-2, IFN- γ ,

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2002 S. Karger AG, Basel
1018–2438/02/1281–0033\$18.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. G. Boltz-Nitulescu
Institute of Pathophysiology
AKH–3Q, Währinger Gürtel 18–20
A–1090 Vienna (Austria)
Tel. +43 1 40400 5103, Fax +43 1 40400 5130, E-Mail georg.boltz@akh-wien.ac.at

TNF- α , and IL-12 [1–3]. The Th2-biased cells, on the other hand, preferentially produce IL-4, IL-5, IL-6, IL-10 and IL-13 [1, 2]. Functionally, Th1 responses mediate delayed-type hypersensitivity reactions, activate macrophages and regulate the production of IgG_{2a} in mice; these events are essential for combating infections by intracellular bacteria, protozoa and viruses. Th1-type responses are further involved in some autoimmune diseases, in contact dermatitis, allograft rejection, and in some chronic inflammatory disorders [4]. Th2 cells participate in humoral responses and protective immunity to extracellular pathogens by promoting B cell activation and antibody production [3–5]. Th1 and Th2 clones may also differ in their requirements of antigen presentation, since B cells preferentially stimulate Th2 clones, whereas macrophage and dendritic cells preferentially stimulate Th1 clones [3–5].

Patterns of cytokine expression are controlled by a complex regulatory network involving cytokines, eicosanoids, glucocorticoids and vitamins [6]. The effects of 1 α ,25-dihydroxyvitamin D₃ (calcitriol; 1 α ,25-dihydroxycholecalciferol), the most potent metabolite of vitamin D₃, on cytokine production and gene expression in mononuclear phagocytes and lymphocytes are not well defined. In human peripheral blood mononuclear cells (PBMC), calcitriol increases the amount of IL-1 detected in culture supernatants after 3 days, and diminishes IL-2 production [7]. In the human monocytoid cell line U937, calcitriol induces cell maturation and the production of IL-1 protein [8, 9] and IL-1-specific mRNA [9–11]. In these cells, as well as in PBMC, calcitriol induces the expression of IL-6 and TNF- α [8–10]. Calcitriol decreases the production of IL-2 and IFN- γ by activated human T lymphocytes as well as T cell proliferation [12–14]. In T cells from mice with experimental autoimmune encephalomyelitis, calcitriol markedly increases the level of IL-4 and TGF- β transcripts [15]. Recently, it has been shown that calcitriol affects Th cell polarization by inhibiting Th1 and augmenting Th2 cell development in mice [6].

The main purpose of this study was to evaluate and compare the influence of calcitriol on the patterns of cytokine production by human PBMC and human Th1 and Th2 cell clones specific for Bet v 1, the major birch (*Betula verucosa*) pollen allergen [16]. Our results show that calcitriol may alter the cytokine profile secretion by inhibiting Th1 type cytokines (IL-2 and IFN- γ) in both human PBMC and Th1 cell clones. The production of IL-4 was suppressed in Bet v 1-specific T cell clones but not in PBMC.

Materials and Methods

Isolation and Culture of PBMC

Heparinized venous blood was collected from healthy volunteers after informed consent. PBMC were obtained by Ficoll-Hypaque (Pharmacia Biotech Inc., Uppsala, Sweden) density-gradient centrifugation. Cells from the interphase were harvested, washed and resuspended in RPMI-1640 culture medium (Sigma Chemical Co., Deisenhofen, Germany), supplemented with 2 mM L-glutamine, 2.5 mg/ml ciprofloxacin (Bayer AG, Leverkusen, Germany) and 10% heat-inactivated fetal calf serum (FCS; Seromed, Linz, Austria).

PBMC were cultured at 37°C at a density of 2×10^6 cells/ml in 6- or 24-well plates (Costar, Cambridge, Mass., USA) in a fully humidified air atmosphere containing 5% CO₂, in the presence or absence of calcitriol [17]. Calcitriol, kindly supplied by F. Hoffmann-La Roche Ltd. (Basel, Switzerland), was dissolved in 95% ethanol as a stock solution of 10^{-4} M, and stored at –70°C. Before use it was diluted with a medium containing 10% FCS [18, 19].

Influence of Calcitriol on PHA-Driven T Cell Proliferation and Cytokine Production

PBMC were resuspended in complete RPMI-1640 medium supplemented with 1% v/v phytohemagglutinin (PHA; Life Technologies, Grand Island, N.Y., USA) and cultured for 72 h at 37°C in 96-well flat-bottom microtiter plates in the absence or presence of various concentrations of calcitriol. The cells were pulsed with 0.5 μ Ci/well ³H-thymidine (Sigma) for the last 16 h. Thereafter, the cells were harvested and the amount of ³H-thymidine incorporated into DNA was assessed by liquid scintillation.

To induce cytokine production, PBMC were stimulated with phorbol-12-myristate-13-acetate (PMA, 10 ng/ml) and ionomycin (1.25 μ M), both purchased from Sigma. Cells were harvested after 6, 12, 24, 48 and 72 h of culture, and cell-free supernatants were aliquoted and frozen once recovered after centrifugation. Trypan blue exclusion revealed a cell viability greater than 98% for freshly isolated cells. The viability of PBMC cultured for 3 days in the presence of calcitriol varied between 92.7 and 96.3%, as demonstrated by FACS analysis of propidium-iodide-stained cells [19].

Allergen-Specific Human T Cell Clones

T cell clones specific for Bet v 1 allergen were generated and characterized as described previously [16]. Th clones, classified as Th0, Th1 or Th2 according to their cytokine production pattern, were incubated in 24-well plates at a density of 1×10^6 cells/ml with irradiated (600 rad) autologous antigen-presenting cells (APC), and cultured for 24 h in the presence of recombinant Bet v 1 allergen (10 μ g/ml; Biomay, Vienna, Austria), and/or calcitriol. In other experiments, Th clones were stimulated with anti-CD3 monoclonal antibodies (mAb) OKT3 (Coulter, Miami, Fla., USA) and PMA (10 ng/ml) and cultured with or without calcitriol.

Measurement of Cytokines in Cell Culture Supernatants

Cell-free culture supernatants were harvested, aliquoted and frozen at –70°C. Cytokine levels were measured by enzyme-amplified sensitivity immunoassays (EASIA) or enzyme-linked immunosorbent assay (ELISA) kits obtained from BioSource International (Camarillo, Calif., USA), and quantified with an ELISA reader (Dynatech MR-7000, Dynal Incorporation, Great Neck, N.Y., USA) at 450/490 nm [20]. A standard curve for each assay was generated, and cytokine production was calculated. Sensitivity limits were: 0.03 IU/ml for IFN- γ ; 0.1 U/ml

for IL-2; 1 pg/ml for IL-10; 2 pg/ml for IL-1 β , IL-4, IL-6 and IL-12; 3 pg/ml for TNF- α and GM-CSF, and <4 pg/ml for IL-5.

Detection of Intracellular Cytokines

Human PBMC (10^6 /ml) were cultured in Ultra Culture Medium (Bio Whittaker, Walkersville, Md., USA) supplemented with 2 mM L-glutamine, 170 mg/l gentamicin sulfate (Sigma) and 1% v/v PHA. After 3 days, the culture medium was replaced with fresh medium supplemented with IL-2 (20 U/ml, La Roche) and calcitriol (10^{-8} M). On day 7, cells were washed once, IL-2 and calcitriol were replaced, and cells were cultured for another 3 days. Cultures with IL-2 alone served as control.

Expression of IFN- γ , IL-2, and IL-4 was assessed at single-cell level by flow cytometry [21]. At the respective time points, cells were stimulated with 10 ng/ml PMA and 1.25 μ M ionomycin in the presence of 2 μ M monensin (all from Sigma). After 4 h, cells were harvested, washed and fixed with 2% formaldehyde. Immunofluorescence staining was performed with fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated anti-human mAb: anti-IFN- γ (FITC), anti-IL-2 (PE) and anti-IL-4 (PE), all purchased from Pharmingen (San Diego, Calif., USA), and the respective isotype controls. At least 10^4 cells were acquired on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, Calif., USA). Lymphocytes were gated according to their light scatter characteristics (forward scatter, side scatter) and further analyzed for cytokine expression using CellQuest software (Becton Dickinson).

Statistics

The data are expressed as the mean $\pm$ SD. Statistical analysis between groups was determined by an unpaired Student t test. A level of $p < 0.05$ was considered statistically significant.

Results

T Cell Proliferation

The results depicted in figure 1 show that calcitriol inhibited PHA-driven T cell proliferation in a concentration-dependent manner. Approximately 70% inhibition was obtained with calcitriol at a concentration of 10^{-7} M.

Regulation of Cytokine Secretion by PBMC

PBMC were cultured for 2 days with various concentrations of calcitriol and cytokine production measured in cell culture supernatants. As shown in figure 2, the secretion of IL-2 and IFN- γ was inhibited by calcitriol in a concentration-dependent manner. By contrast, the amount of IL-10 was evidently increased by calcitriol. The production of IL-4 was not significantly modulated by calcitriol at any concentration.

Time-Course Effects of Calcitriol on Cytokine Secretion by PBMC

In time-course experiments, the effect of calcitriol on IL-2 and IL-4 secretion was detectable after 6 h of treat-

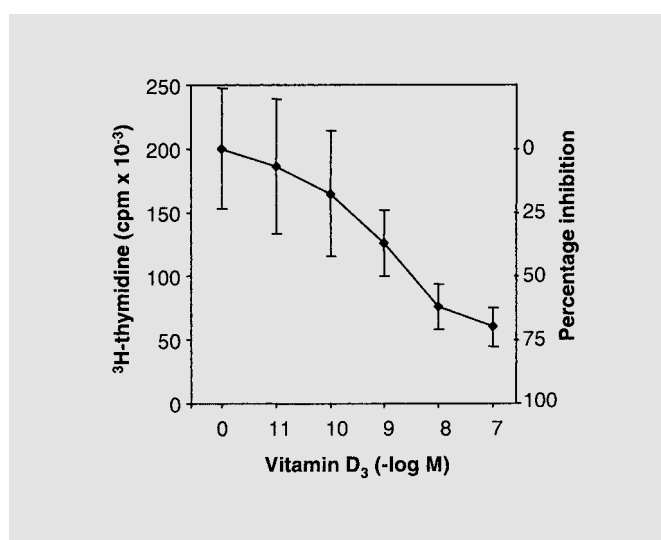


Fig. 1. PBMC were cultured in complete RPMI-1640 medium supplemented with PHA (1% v/v) for 72 h at 37°C in 96-well flat-bottom microtiter plates (3×10^5 cells per well) in the absence or presence of various concentrations of calcitriol. For the last 16 h, cells were pulsed with ^{3} H-thymidine (0.5 μ Ci/well), then the cells were harvested and the amount of ^{3} H-thymidine assessed by liquid scintillation. Data from 6 experiments, performed in triplicates, are presented as mean $\pm$ SD.

ment and reached a maximum at 48 h (fig. 3). Significant levels of IFN- γ and IL-10 were measured after 48 and 12 h, respectively. As figure 3 further illustrates, calcitriol at 10^{-7} M significantly reduced the IL-2 and IFN- γ secretion after 24 or 48 h treatment, respectively. Interestingly, the production of IL-10, but not of IL-4, was upregulated by calcitriol.

Regulation of Other Cytokine Expression in PBMC

The influence of various concentrations of calcitriol on the secretion of other cytokines is shown in table 1. Calcitriol at a concentration between 10^{-7} and 10^{-9} M inhibited the production of GM-CSF, TNF- α and IL-12 in a dose-dependent manner. The synthesis of IL-6 and IL-1 β was weakly diminished, whereas IL-5 secretion was slightly induced.

Detection of Intracellular Cytokines in PBMC

The effect of calcitriol on the number of cytokine-producing cells was analyzed by FACS. As illustrated in figure 4, the percentage of CD4⁺ T cells containing IL-2, IFN- γ , or both, was significantly diminished by calcitriol. In contrast, calcitriol did not induce changes in the number of IL-4-producing Th cells.

Fig. 2. Influence of various concentrations of calcitriol on cytokine production by PBMC. Cells were stimulated with PMA (10 ng/ml) and ionomycin (1.25 μ M) and cultured for 2 days in the absence or presence of indicated concentrations of calcitriol. Supernatants were collected and cytokines measured by ELISA. Data from 5–8 experiments are presented as mean $\pm$ SD. Statistically significant differences, Student's t test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

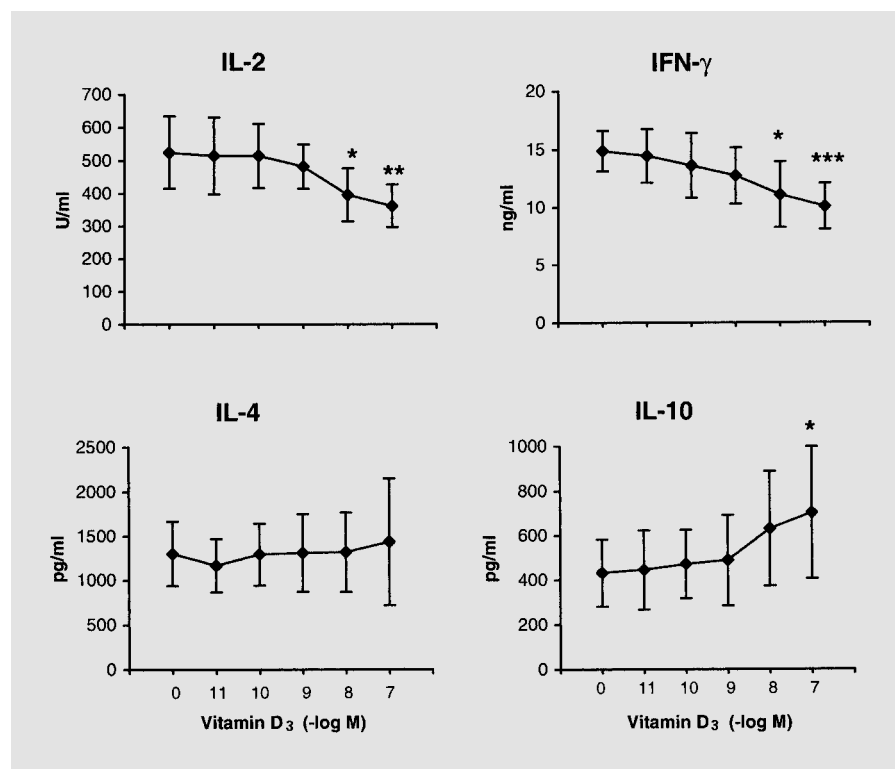


Fig. 3. Time kinetics. Cells were stimulated with PMA (10 ng/ml) and ionomycin (1.25 μ M) and cultured for the indicated time periods in tissue culture medium alone (◆) or supplemented with 10^{-7} M (▲) or 10^{-9} M (■) calcitriol. Supernatants were collected and cytokines measured by ELISA. Data from 3 experiments are presented as mean $\pm$ SD. Statistically significant differences, Student's t test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

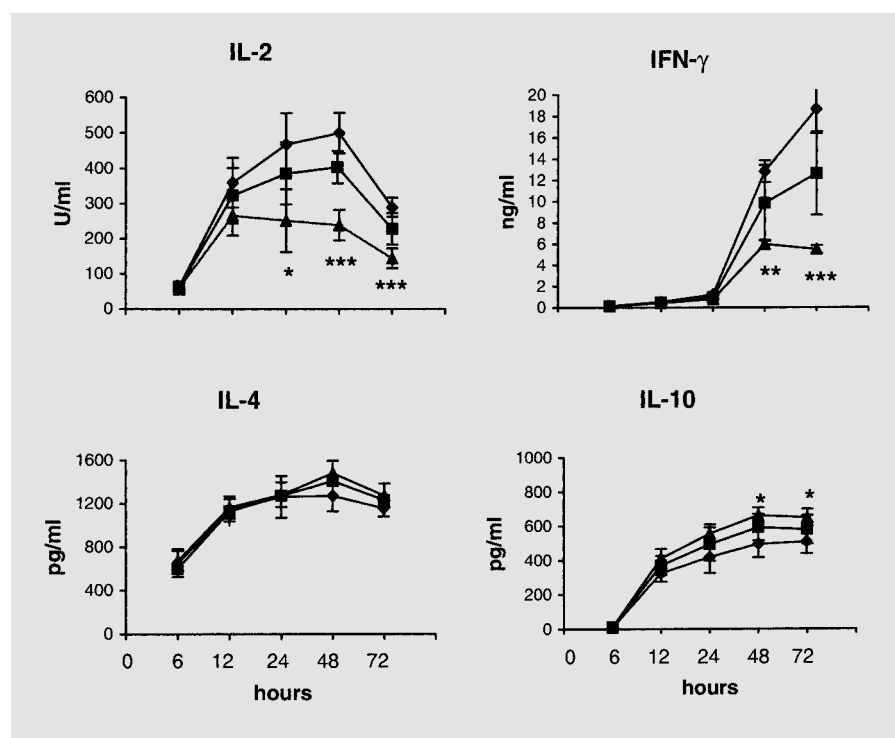


Fig. 4. Intracellular detection of cytokines in PBMC. PBMC from healthy donors ($n = 10$) were cultured with or without $1\alpha,25(\text{OH})_2\text{D}_3$ for 14 days as described in Materials and Methods. Percentages of cytokine-producing cells were determined by flow cytometry. Cells were previously gated as lymphocytes according to their light scatter characteristics, and thereafter analyzed for expression of IFN- γ (x-axis) and IL-2 or IL-4 (y-axis). Dot plots from one representative experiment are shown. Values within quadrants represent means of 10 experiments $\pm$ SD. Asterisks indicate significant difference between cultures with calcitriol (D_3) and control (Co): * $p < 0.05$; ** $p < 0.01$.

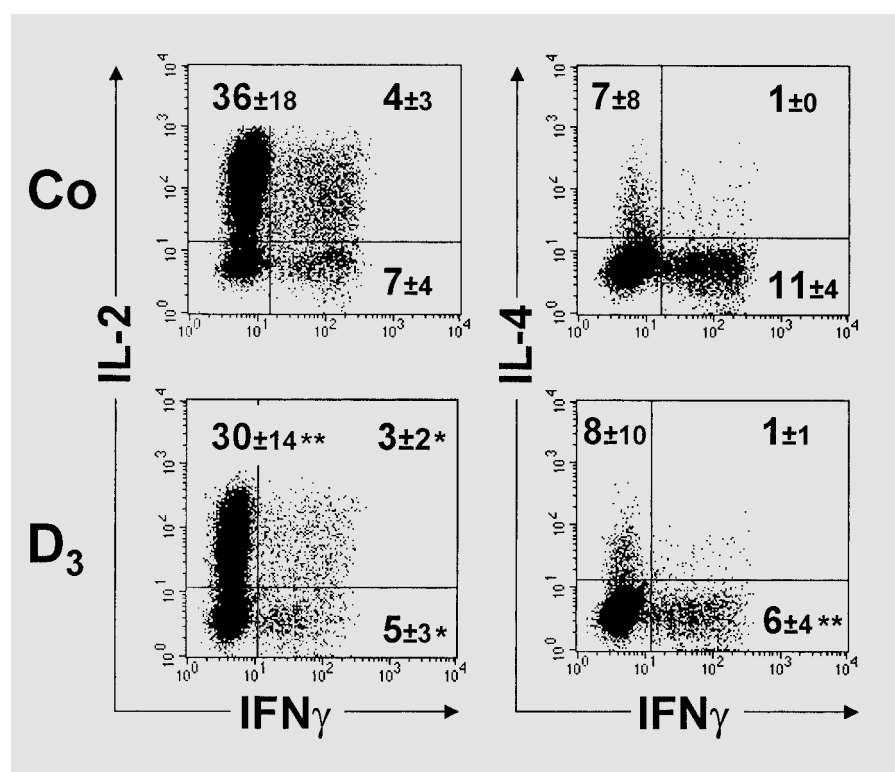


Table 1. The influence of calcitriol on cytokine production by human PBMC

Calcitriol -log M	IL-1 β ng/ml	IL-5 pg/ml	IL-6 ng/ml	IL-12 pg/ml	GM-CSF pg/ml	TNF- α ng/ml
None	1,025 $\pm$ 163	163 $\pm$ 68	46.5 $\pm$ 8.5	2,068 $\pm$ 259	518 $\pm$ 101	7.7 $\pm$ 2.2
7	864 $\pm$ 106*	251 $\pm$ 61*	31.8 $\pm$ 9.3*	862 $\pm$ 218***	358 $\pm$ 37***	3.6 $\pm$ 0.9***
8	872 $\pm$ 131*	249 $\pm$ 53*	33.5 $\pm$ 8.2*	1,247 $\pm$ 358***	367 $\pm$ 35***	4.4 $\pm$ 1.5**
9	1,072 $\pm$ 146	209 $\pm$ 87	42.5 $\pm$ 7.4	1,667 $\pm$ 453*	398 $\pm$ 52*	5.3 $\pm$ 1.2*
10	1,120 $\pm$ 150	163 $\pm$ 53	44.5 $\pm$ 12.5	1,881 $\pm$ 420	435 $\pm$ 105	7.1 $\pm$ 2.2
11	1,171 $\pm$ 152	164 $\pm$ 59	48.3 $\pm$ 9.6	1,991 $\pm$ 427	492 $\pm$ 114	7.8 $\pm$ 21.9

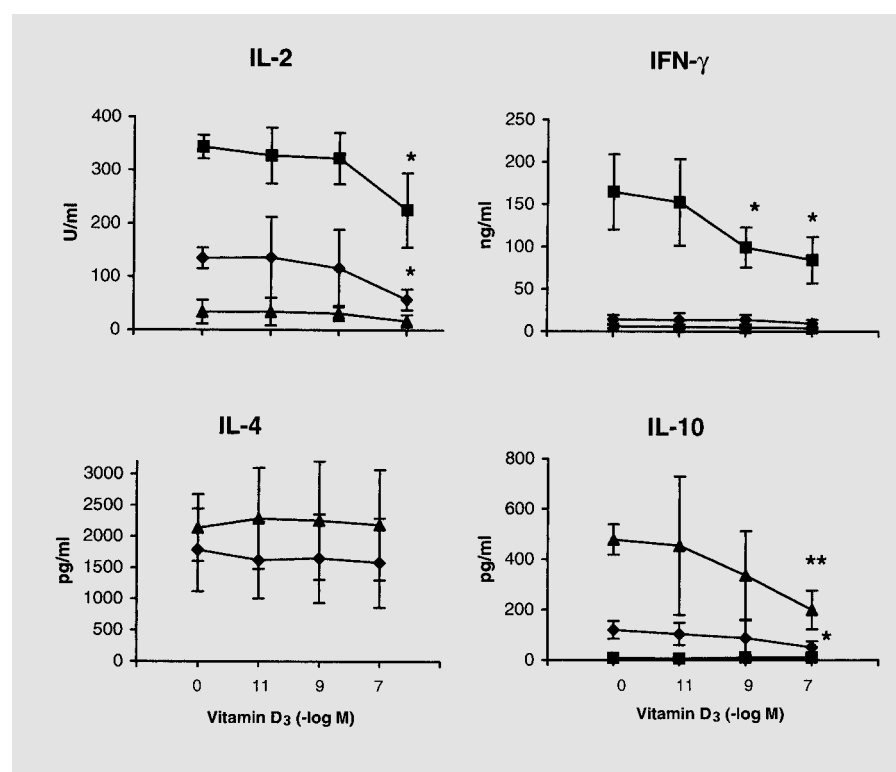
PBMC isolated from apparently healthy donors were cultured for 48 h with lipopolysaccharide (100 ng/ml; for IL-1 β , IL-6 and TNF- α production), or PHA (20 μ g/ml; for IL-5, GM-CSF and IL-12 secretion) in the absence or presence of calcitriol. The supernatants were harvested, aliquoted and frozen. The individual cytokines were measured by cytokine-specific EASIA and the data from 6 separate experiments are presented as mean $\pm$ SD. Statistically significant differences versus control, Student's t test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

Effects of Calcitriol on Cytokine Production by Th Cell Clones

In further experiments, the effects of calcitriol on cytokine production by human CD4 $^{+}$ T cell clones was investigated. The results from figure 5 show that calcitriol inhibited the production of IL-2 and IFN- γ by Bet v 1-spe-

cific Th1 clones stimulated by Bet v 1 allergen in a dose-dependent manner. In contrast to data obtained with PBMC, the Bet v 1-induced secretion of IL-10 by Th2 clones was markedly decreased by calcitriol, whereas IL-4 synthesis was not significantly altered.

Fig. 5. Influence of calcitriol on cytokine production by Th cell clones. Bet v 1-specific human Th0 (◆), Th1 (■) or Th2 (▲) cell clones were incubated with irradiated (600 rad) autologous APC, and cultured for 24 h with recombinant Bet v 1 (10 µg/ml) in the presence or absence of calcitriol. Supernatants were collected and cytokines measured by ELISA. Data from 6–9 experiments are presented as mean ± SD. Statistically significant differences, Student's t test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.



Discussion

The Th1 and Th2 cells develop from naive CD4⁺ T cells, and the differentiation process is initiated by the T cell receptor binding and directed by the cytokine environment. IL-4 is required for Th2 development [22], whereas IL-12 plays a pivotal role in the production of Th1 cells and the inhibition of Th2 responses [23]. The precise mechanisms underlying selective induction of Th1 or Th2 cells are still not fully understood. In the regulation of the Th1/Th2 balance, several transcription factors have been described. The transcription factor *T-bet* induces the expression of IFN-γ and suppresses the production of IL-4 and IL-5 [24], whereas *c-maf* and GATA-3 increase the expression of IL-4 and inhibit differentiation of CD4⁺ cells into Th1 subset [25, 26]. The expression of GATA-3 is upregulated by IL-4, which in turn inhibits the expression of IL-12 receptor β₂ chain, and subsequently blocks the development and commitment of Th1 cells [26]. Cells that develop along the Th1 pathway contain both β₁ and β₂ subunits of IL-2 receptor, but there is a selective loss of the β₂ chain during differentiation into Th2 cells [27].

In the present study, calcitriol significantly inhibited the secretion of IL-2 and IFN-γ by PBMC as well as by Th1 clones, and reduced the percentage of CD4⁺ cells producing IL-2 and IFN-γ. Furthermore, the synthesis of IL-12, a Th1-biased cytokine, by PBMC was markedly diminished by calcitriol. Most likely, this effect is caused by the inhibition of NF-κB activation and transcriptional repression of the IL-12p40 gene [28].

The production of IL-5 and IL-10 by PBMC was significantly augmented by calcitriol. These results are in line with recent data showing that calcitriol acts directly on Th cells from BALB/c and C57BL/6 mice by enhancing IL-4, IL-5, and IL-10 production [6]. In our experiments we did not find a significant increase either in the total amount of IL-4 secreted in PBMC culture supernatants or in the percentage of CD4⁺ cells containing intracytoplasmic IL-4. It should be emphasized that calcitriol upregulated the production of IL-10 in PBMC, but not in Th2 cell clones. Initially, IL-10 was identified as a Th2 product that inhibited Th1 cell proliferation by downregulating IFN-γ and IL-2 synthesis [29]. Therefore the increased IL-10 production by PBMC may be responsible for reduced IL-2 synthesis and T cell proliferation. In addition, IL-10 downregulates Th1 cells indirectly by affecting HLA-DR and

CD4 expression and APC function, and diminishes IFN- γ synthesis [18, 29].

In PBMC preparations, some B cells, monocytes and dendritic cells are present, and following activation, these cells are able to produce IL-10 [29]. Calcitriol has the ability to inhibit cytokine-driven differentiation of human monocytes into dendritic cells, their maturation, activation and survival [30, 31]. Furthermore, calcitriol markedly inhibited the upregulation of costimulatory molecules CD40, CD80, CD83 and CD86, and of MHC class II antigens. These events were associated with a reduced capacity of dendritic cells to activate alloreactive T cells in the classic mixed-leukocyte cultures, as well as with decreased IFN- γ and IL-12 secretion and strongly enhanced IL-10 production [30]. On monocytes and monocytic cell lines, calcitriol modulates the expression of Fc receptor for IgG, IgE and IgA, complement receptors, MHC molecules, and other cell surface antigens [18, 19, 32].

The secretion of IL-2, IFN- γ and of IL-10 was consistently diminished in calcitriol-treated Th1 and Th2 cell clones stimulated by Bet v 1 allergen and cultured in the presence of irradiated autologous APC. Similar results, or even higher levels of cytokine production, were observed when Th cell clones were stimulated with anti-CD3 mAb and PMA (data not shown).

It has been previously shown that calcitriol inhibits stem cell factor-dependent mast cell proliferation and the histamine release induced by calcium ionophore A23187 [33], and that it downregulates the IgE-mediated cutaneous reaction [34]. Delayed-type hypersensitivity reaction to dinitrobenzene was impaired in vitamin D-deficient mice supplemented with calcitriol [35]. The immunosuppressive effects of calcitriol and its analogs have been further strengthened by their properties to prevent systemic lupus erythematosus in *lpr/lpr* mice [36] and autoimmune diabetes in nonobese diabetic mice [37]. In experimental studies, calcitriol greatly reduced the incidence and severity of encephalomyelitis [38], and administration of calcitriol in mice increases the level of transcripts for two anti-encephalitogenic cytokines IL-4 and TGF- β several fold [15]. Furthermore, calcitriol and derivatives prolong the survival of allografts [39], and are potentially useful for the treatment of psoriasis [40] and as alternatives to conventional suppressive therapies of some tumors [41].

In previous studies, calcitriol inhibited mitogen-induced T lymphocyte proliferation [13, 42]. It was further shown that the CD45RO⁺ subset of Th cells is more sensitive to the inhibitory effects of calcitriol than the

CD45RA⁺ subset [43]. In our experiments, calcitriol inhibited the ³H-thymidine incorporation into PHA-stimulated PBMC in a concentration-dependent manner. This anti-proliferative effect may be caused by reduced production of T cell-activating cytokines (IL-1 and TNF- α), and by direct action on T cell subsets, primarily through inhibiting IL-2 and IL-12 production.

The biological properties of calcitriol are mediated via binding to a nuclear receptor complex named vitamin D₃ receptor (VDR). The VDR has a ubiquitous tissue distribution, including peripheral blood monocytes, lymphocytes, keratinocytes, fibroblasts, endothelial cells, myelomonocytic cell lines, and some tumor cells [44, 45]. B lymphocytes express a very low number of VDR, whereas naive and activated CD8⁺ T cells express approximately twice as much VDR as CD4⁺ cells [46]. Due to increased receptor longevity and stability, the constitutive VDR expression on both CD4⁺ and CD8⁺ T cells is markedly augmented by calcitriol [46].

Our data indicated that calcitriol preferentially inhibits the production of Th1-type cytokines by PBMC as well as by allergen-specific Th cell clones. They suggest that application of calcitriol, and particularly of its noncalcemic derivatives, may be a useful alternative to conventional therapies for the treatment of Th1-mediated diseases.

Acknowledgements

This work was supported by grants from the 'Hochschuljubiläumsstiftung der Stadt Wien' (H296/96), and by Austrian Science Research Funds, Project No. P11728-MED. We are grateful to Mrs. Magdolna Vermes and Margit Anglmayer for their expert technical assistance.

References

- Mosmann TR, Coffman RL: Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–173.
- Gajewski TF, Pinnas M, Wong T, Fitch FW: Murine Th1 and Th2 clones proliferate optimally in response to distinct antigen-presenting cell populations. *J Immunol* 1991;146:1750–1758.
- Del Prete GF, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S: Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993;150:353–360.
- Romagnani S: Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994;12:227–257.
- Müller K, Jaunin MF, Masouyé I, Saurat J-H, Hauser C: Th2 cells mediate IL-4-dependent local tissue inflammation. *J Immunol* 1993;15:5576–5584.
- Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A: 1 α ,25-Dihydroxyvitamin D₃ has a direct effect on naive CD4⁺ T cells to enhance the development of Th2 cells. *J Immunol* 2001;167:4974–4980.
- Bhalla AK, Amento EP, Krane SM: Differential effects of 1,25-dihydroxyvitamin D₃ on human lymphocytes and monocytes/macrophages: Inhibition of interleukin-2 and augmentation of interleukin-1 production. *Cell Immunol* 1986;98:31–322.
- Amento EP, Bhalla AK, Kurnick JT, Kradin RL, Clemens TL, Holick SA, Holick MF, Krone SM: 1 α ,25-dihydroxyvitamin D₃ induces maturation of the human monocyte cell line U937 and, in association with a factor from human T lymphocytes, augments production of the monokine, mononuclear cell factor. *J Clin Invest* 1984;73:731–739.
- Taimi M, Defacque H, Commes T, Favero J, Caron E, Marti J, Dornand J: Effect of retinoic acid and vitamin D on the expression of interleukin-1 β , tumour necrosis factor- α and interleukin-6 in the human monocytic cell line U937. *Immunology* 1993;79:229–235.
- Bhalla AK, Paavonen T, Williams MM, Delves PJ, Lydyard PM: Regulation of interleukin-1 and tumour necrosis factor gene expression in myelomonocytic cell lines by 1,25-dihydroxyvitamin D₃. *Immunology* 1991;72:61–64.
- Blifeld C, Pehn JL, Jordan SC: Stimulus-specific 1,25(OH)₂D₃ modulation of TNF and IL-1-beta gene expression in human peripheral blood mononuclear cells and monocytoid cell lines. *Transplantation* 1991;51:498–503.
- Reichel H, Koeffler HP, Tobler A, Norman AW: 1 α ,25-Dihydroxyvitamin D₃ inhibits γ -interferon synthesis by normal human peripheral blood lymphocytes. *Proc Natl Acad Sci USA* 1987;84:3385–3389.
- Rigby WFC, Denome S, Fanger MW: Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D₃. Specific inhibition at the level of messenger RNA. *J Clin Invest* 1987;79:1659–1664.
- Lemire JM, Archer DC, Beck L, Spiegelberg HL: Immunosuppressive actions of 1,25-dihydroxyvitamin D₃: Preferential inhibition of Th1 functions. *J Nutr* 1995;125:1704S–1708S.
- Cantorna MT, Woodward WD, Hayes CE, DeLuca HF: 1,25-dihydroxyvitamin D₃ is a positive regulator for the two anti-encephalitogenic cytokines TGF- β 1 and IL-4. *J Immunol* 1998;160:5314–5319.
- Ebner C, Szépfalusi Z, Ferreira F, Jilek A, Valenta R, Parronchi P, Maggi E, Romagnani S, Scheiner O, Kraft D: Identification of multiple T cell epitopes on Bet v 1, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 1993;150:1047–1054.
- Spittler A, Winkler S, Götzinger P, Oehler R, Willheim M, Tempfer C, Weigel G, Függer R, Boltz-Nitulescu G, Roth E: Influence of glutamine on the phenotype and function of human monocytes. *Blood* 1995;86:1564–1569.
- Spittler A, Willheim M, Leutmezer F, Öhler R, Krugluger W, Reissner C, Lucas T, Brodowicz T, Roth E, Boltz-Nitulescu G: Effects of 1 α ,25-dihydroxyvitamin D₃ and cytokines on the expression of MHC antigens, complement receptors and other antigens on human blood monocytes and U937 cells: Role in cell differentiation, activation and phagocytosis. *Immunology* 1997;90:286–293.
- Boltz-Nitulescu G, Willheim M, Spittler A, Leutmezer F, Tempfer C, Winkler S: Modulation of IgA, IgE and IgG Fc receptor expression on human mononuclear phagocytes by 1 α ,25-dihydroxyvitamin D₃ and cytokines. *J Leukoc Biol* 1995;58:256–262.
- Spittler A, Reissner CM, Oehler R, Gornikiewicz A, Gruenberger T, Manhart N, Brodowicz T, Mittelboeck M, Boltz-Nitulescu G, Roth E: Immunomodulatory effects of glycine on LPS-treated monocytes: Reduced TNF- α production and accelerated IL-10 expression. *FASEB J* 1999;13:563–571.
- Willheim M, Thien R, Schratlbauer K, Bajna E, Holub M, Gruber R, Baier K, Pietschmann P, Reinisch W, Scheiner O, Peterlik M: Regulatory effects of 1 α ,25-dihydroxyvitamin D₃ on the cytokine production of human peripheral blood lymphocytes. *J Clin Endocrinol Metab* 1999;84:3739–3744.
- Abbas AK, Murphy KM, Sher A: Functional diversity of helper T lymphocytes. *Nature* 1996;383:787–793.
- Mattner F, Smioldo S, Galibati F, Muller M, Di Lucia P, Poliani PL, Martino G, Panina-Bordignon P, Adorini L: Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D₃. *Eur J Immunol* 2000;30:498–508.
- Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH: A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000;100:655–669.
- Ho IC, Hodge MR, Rooney JW, Glimcher LH: The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell* 1996;85:973–983.
- Gorelik L, Fields PE, Flavell RA: Cutting edge: TGF- β inhibits Th type 2 development through inhibition of GATA-3 expression. *J Immunol* 2000;165:4773–4777.
- Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH: The interleukin-12/interleukin-12-receptor system: Role in normal and pathologic immune responses. *Annu Rev Immunol* 1998;16:495–521.
- D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di-Lucia P, Lang R, Sinigaglia F, Panina-Bordignon P: Inhibition of IL-12 production by 1,25-dihydroxyvitamin D₃. Involvement of NF- κ B downregulation in transcriptional repression of the p40 gene. *J Clin Invest* 1998;101:252–262.
- Mosmann TR: Properties and functions of interleukin-10. *Adv Immunol* 1994;56:1–26.
- Penna G, Adorini L: 1 α ,25-Dihydroxyvitamin D₃ inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol* 2000;164:2405–2411.
- Piemonti L, Monti P, Sironi M, Fraticelli P, Leone BE, Cin ED, Allavena P, Di Carlo V: Vitamin D₃ affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J Immunol* 2000;164:4443–4451.
- Öberg F, Botling J, Nilsson K: Functional antagonism between vitamin D₃ and retinoic acid in the regulation of CD14 and CD23 expression during monocytic differentiation of U-937 cells. *J Immunol* 1993;150:3487–3495.
- Toyota N, Sakai H, Takahashi H, Hashimoto Y, Iizuka H: Inhibitory effect of 1 α ,25-dihydroxyvitamin D₃ on mast cell proliferation and A23187-induced histamine release, also accompanied by a decreased c-kit receptor. *Arch Dermatol* 1996;288:709–715.
- Katayama I, Minatohara K, Yokozeki H, Nishioka K: Topical vitamin D₃ downregulates IgE-mediated murine biphasic cutaneous reactions. *Int Arch Allergy Immunol* 1999;111:71–76.
- Yang S, Smith C, Prah JM, Luo X, DeLuca HF: Vitamin D₃ deficiency suppress cell-mediated immunity in vivo. *Arch Biochem Biophys* 1993;303:98–106.

- 36 Koizumi T, Nakao Y, Matsui T, Nakagawa T, Matsuda S, Komoriya K, Kanai Y, Fujita T: Effects of corticosteroid and 1,24R-dihydroxyvitamin D₃ administration on lymphoproliferation and autoimmune disease in MRL/MP-*lpr/lpr* mice. *Int Arch Allergy Appl Immunol* 1985;77:396–404.
- 37 Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R: Prevention of autoimmune diabetes in NOD mice by 1,25-dihydroxyvitamin D₃. *Diabetologia* 1994;37:552–558.
- 38 Cantorna MT, Hayes C, DeLuca HF: 1,25-Dihydroxyvitamin D₃ reversibly blocks the progression of relapsing encephalomyelitis with a subset of T lymphocytes: A model of multiple sclerosis. *Proc Soc Acad Sci USA* 1996;93:7861–7864.
- 39 Hullett DA, Cantorna MT, Redaelli C, Humal-Winter J, Hayes CE, Sollinger HW, DeLuca HF: Prolongation of allograft survival by 1,25-dihydroxyvitamin D₃. *Transplantation* 1998;66:824–828.
- 40 Kragballe K, Gjertsen BT, De Hoop D, Karlsmark T, van de Kerkhof PC, Larko O, Nieboer C, Roed-Petersen J, Strand A, Tikjob G: Double-blind, right/left comparison of calcipotriol and betamethasone valerate in treatment of psoriasis vulgaris. *Lancet* 1991;337:193–196.
- 41 Van den Bemd GJ, Pols HA, van Leeuwen JP: Anti-tumor effects of 1,25-dihydroxyvitamin D₃ and vitamin D₃ analogs (review). *Curr Pharm Des* 2000;6:717–732.
- 42 Lemire JM, Adams JS, Kermani-Arab V, Bakke AC, Sakai R, Jordan SC: 1,25-Dihydroxyvitamin D₃ suppresses human T helper/inducer lymphocyte activity in vitro. *J Immunol* 1985;134:3032–3035.
- 43 Müller K, Bendtzen K: Inhibition of human T lymphocyte proliferation and cytokine production by 1,25-dihydroxyvitamin D₃. Differential effects on CD45RA⁺ and CD45RO⁺ cells. *Autoimmunity* 1992;14:37–43.
- 44 Pike JW: Vitamin D₃ receptors: Structure and function in transcription. *Annu Rev Nutr* 1991;11:189–216.
- 45 DeLuca HF, Cantorna MT: Vitamin D: Its role and use in immunology. *FASEB J* 2001;15:2579–2585.
- 46 Wiese RJ, Uhland-Smith A, Ross TK, Prah JM, DeLuca HF: Up-regulation of the vitamin D receptor in response to 1,25-dihydroxyvitamin D₃ results from ligand-induced stabilization. *J Biol Chem* 1992;267:20082–20086.