



Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: [www.elsevier.com/locate/ybbrc](http://www.elsevier.com/locate/ybbrc)



# 1,25-dihydroxyvitamin D<sub>3</sub> impairs NF-κB activation in human naïve B cells

Kerstin Geldmeyer-Hilt<sup>a</sup>, Guido Heine<sup>a,b</sup>, Björn Hartmann<sup>a</sup>, Ria Baumgrass<sup>b</sup>, Andreas Radbruch<sup>b</sup>, Margitta Worm<sup>a,\*</sup>

<sup>a</sup> Allergie-Centrum-Charité, CCM, Klinik für Dermatologie und Allergologie, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

<sup>b</sup> Deutsches Rheuma-Forschungszentrum Berlin, Charitéplatz 1, 10117 Berlin, Germany

## ARTICLE INFO

### Article history:

Received 11 March 2011

Available online 21 March 2011

### Keywords:

Vitamin D

Calcitriol

Vitamin D receptor

NF-κB

Naïve B cells

## ABSTRACT

1α,25-dihydroxyvitamin D<sub>3</sub> (calcitriol), the bioactive metabolite of vitamin D, modulates the activation and inhibits IgE production of anti-CD40 and IL-4 stimulated human peripheral B cells. Engagement of CD40 results in NF-κB p50 activation, which is essential for the class switch to IgE. Herein, we investigated by which mechanism calcitriol modulates NF-κB mediated activation of human naïve B cells. Naïve B cells were predominantly targeted by calcitriol in comparison with memory B cells as shown by pronounced induction of the VDR target gene *cyp24a1*. Vitamin D receptor activation resulted in a strongly reduced p105/p50 protein and mRNA expression in human naïve B cells. This effect is mediated by impaired nuclear translocation of p65 and consequently reduced binding of p65 to its binding site in the p105 promoter. Our data indicate that the vitamin D receptor reduces NF-κB activation by interference with NF-κB p65 and p105. Thus, the vitamin D receptor inhibits costimulatory signal transduction in naïve B cells, namely by reducing CD40 signaling.

© 2011 Elsevier Inc. All rights reserved.

## 1. Introduction

The biologically active form of vitamin D (1,25-dihydroxyvitamin D<sub>3</sub>, calcitriol) mediates most of its functions through activating the nuclear vitamin D receptor (VDR) and regulation of its target genes. About 0.5% (approximately 200 genes) of all genes in the human genome are estimated to be transcriptionally regulated by the VDR. However, by various mechanisms the VDR seems to be involved in the indirect regulation of even more genes [1]. Besides its important role in calcium homeostasis and bone metabolism, it is of importance in modulating innate and adaptive immunity [2]. The VDR is expressed in several immune cells including B and T cells [3]. Calcitriol has also been shown to have a negative effect on NF-κB activation in various cell types. In murine macrophages nuclear translocation of p65 is inhibited by an upregulation of the inhibitor of kappaBα (IκBα) [4]. In murine embryonic fibroblasts, p65 is retained in the cytosol by direct complex formation with the VDR [5]. Furthermore, the activated VDR plays an important role in modulating the immune system, indicated by the fact that several autoimmune diseases are linked to vitamin

D deficiency, including asthma [6], type 1 diabetes [7] and systemic lupus erythematosus [8].

We have previously shown that calcitriol reduces ε-germline transcript expression, IgE production and nuclear amount of NF-κB p50 after anti-CD40 and IL-4 stimulation in human B cells [9]. p50 is an essential transcription factor for IgE class switch recombination as p50<sup>−/−</sup> mice do not produce IgE [10], whereas isotype switch is enhanced in mice that lack the NF-κB inhibitor IκBα [11]. Hence we hypothesized that VDR activation impairs IgE class switch recombination via inhibition of NF-κB p50. The aim of this study was to elucidate the underlying mechanism of calcitriol mediated modulation of NF-κB activation.

## 2. Materials and methods

### 2.1. Cell preparation and cell culture

After written informed consent and approval by the Charité ethical board, peripheral mononuclear cells were isolated by density gradient centrifugation with ficoll hypaque (Biochrom KG, Berlin, Germany) from blood filters of healthy donors obtained from the Charité blood donation. B cells were purified by magnetic cell sorting using magnetically coupled CD19-multisort beads (Miltenyi Biotec, Bergisch-Gladbach, Germany) as described previously [12]. Naïve B cells were purified by depletion of CD27<sup>+</sup> and CD14<sup>+</sup> cells. Cells were cultured in Advanced RPMI 1640 (Invitrogen, Darmstadt, Germany) supplemented with 5% charcoal-stripped FCS

**Abbreviations:** VDR, vitamin D receptor; ORF, open reading frame; IκBα, inhibitor of kappaBα; ChIP, chromatin immunoprecipitation.

\* Corresponding author. Fax: +49 30 450 518931.

**E-mail addresses:** [kerstin.hilt@charite.de](mailto:kerstin.hilt@charite.de) (K. Geldmeyer-Hilt), [guido.heine@charite.de](mailto:guido.heine@charite.de) (G. Heine), [bjoern.hartmann@charite.de](mailto:bjoern.hartmann@charite.de) (B. Hartmann), [baumgrass@drfz.de](mailto:baumgrass@drfz.de) (R. Baumgrass), [radbruch@drfz.de](mailto:radbruch@drfz.de) (A. Radbruch), [margitta.worm@charite.de](mailto:margitta.worm@charite.de) (M. Worm).

(Invitrogen), 4 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin (all from Biochrom). Cells were stimulated with 1 µg/ml anti-CD40 (Santa Cruz, Heidelberg, Germany) and 10 ng/ml recombinant human IL-4 (Miltenyi Biotec) with/out 100 nM calcitriol (Sigma–Aldrich, Steinheim, Germany). Cultures were carried out at 37 °C in humidified air and 5% CO<sub>2</sub>.

## 2.2. Protein isolation and western blot

Whole cell extracts were prepared using Triton lysis buffer (150 mM NaCl, 1% Triton X-100, 50 mM Tris HCl pH 8.0, all from Sigma–Aldrich). Nuclear extracts were prepared with the NE-PER extraction reagent (Pierce, Rockford, USA) containing a protease inhibitor cocktail (Roche, Mannheim, Germany). Protein concentration was quantified by standard Coomassie plus protein assay (Pierce). Ten microgram whole cell lysate or nuclear extracts were separated in a 12% Tris glycine sodium dodecyl sulphate polyacrylamide gel and proteins were transferred to a polyvinylidene difluoride membrane (Amersham Biosciences, Freiburg, Germany) and blocked for 1 h with 5% milk powder/PBS. The membrane was incubated overnight at 4 °C with 1 µg/ml anti-human NF-κB1 (Lifespan Biosciences, Seattle, WA, USA) in 1% milk powder/PBS or 0.5 µg/ml anti-human p65 (BD, Heidelberg, Germany) in 5% BSA/PBS. After washing the membrane with PBS-Tween, HRP conjugated secondary antibody (Santa Cruz, Heidelberg, Germany) was added for 1 h at room temperature. The antigen detection was performed with the chemiluminescent detection system ECL Plus (Amersham Biosciences).

## 2.3. RNA extraction and quantitative PCR

RNA isolation was performed with the Nucleospin RNA II kit (Macherey–Nagel, Dueren, Germany). For cDNA synthesis TaqMan Reverse Transcription Reagent (Applied Biosystems, Darmstadt, Germany) was used. Quantitative RT-PCR was done as described in [13]. Sequences of specific primers (TIB MOLBIOL, Berlin, Germany) were p105 for CCA gTg AAG ACC ACC TCT CA, p105 rev TgA gTT TgC ggA Agg ATg TC, HPRT for ATC AgA CTg AAG AgC TAT TgT AAT gAC CA, HPRT rev Tgg CTT ATA TCC AAC ACT TCg Tg, Cyp24a1 for Cgg gTg TAC CAT TTA CAA CTC gg and Cyp24a1 rev CTC AAC Agg CTC ATT gTC TgT gg.

## 2.4. Chromatin immunoprecipitation

Naïve B cells were fixed and lysed and magnetic chromatin immunoprecipitation (ChIP) was carried out as previously described [14] with anti-p65 (C-20, Santa Cruz). DNA was purified using NucleoSpin extract II (Macherey–Nagel). The precipitated DNA was quantified by using qPCR. A p65 binding element of the IκBα promoter was amplified as a positive control for p65 binding. *Trpv6* open reading frame (ORF) was used as a negative control. Sequences of specific primers were p105 prom for Tgg ACC gCA TgA CTC TAT CA and rev ggC TCT ggC TTC CTA gCA g, IκBα prom for gAC gAC CCC AAT TCA AAT Cg and rev TCA ggC TCg ggg AAT TTC C, *trpv6* ORF for TgA TgT CCA ggC CCT gAA CAA gT and rev gCT CCG ggg CAG CCT CCA TCA gC.

## 2.5. Flow cytometric analysis of IκBα degradation

Human CD19<sup>+</sup> B cells were stimulated with 10 ng/ml recombinant human IL-4 for 24 h. IκBα degradation was induced by anti-CD40 stimulation for 15, 30, and 45 min. Cells were preincubated with 100 nM calcitriol for 1 h. After fixation with 2% paraformaldehyde for 10 min at 37 °C, cells were stained with anti-CD27 (LG.7F9, eBioscience, San Diego, CA, USA). Cells were then stained intracellularly with anti-IκBα (L35A5, Cell Signaling Technology,

Boston, MA, USA) in 1% saponin/1% BSA/PBS for 30 min at 4 °C and analyzed by flow cytometry. A 30000 gated B cells were collected for each sample and the geometric mean fluorescence for IκBα was analyzed.

## 2.6. Statistical analysis

Data were analyzed with prism 5.0 (GraphPad Software, LaJolla, CA, USA) using the non-parametrical Wilcoxon signed rank test or students *t*-test for matched pairs. Data are shown as median ± SEM. *p* < 0.05 was considered statistically significant.

# 3. Results and discussion

## 3.1. Activated vitamin D receptors inhibit p105 expression in human naïve B cells

To investigate the role of VDR in the regulation of NF-κB p50 and its precursor p105 in B cells, we first identified the B cell subpopulation that is the main target of the natural VDR agonist calcitriol. Naïve (CD27<sup>−</sup>) and memory (CD27<sup>+</sup>) B cells were distinguished according to their surface expression of CD27 [15]. VDR expression was induced with anti-CD40 and IL-4 and the expression of its target gene *cyp24a1* [16] was analyzed after calcitriol incubation. In naïve B cells, *vdr* expression was 3.1 to 7.2-fold higher than in memory B cells, the VDR target-gene *cyp24a1* was induced 4.3 to 8.2-fold stronger in naïve B than in memory B cells (Supplementary table 1). Thus, all following experiments were carried out with naïve CD19<sup>+</sup> CD27<sup>−</sup> B cells.

NF-κB p50 and also p105 protein expression were induced by stimulation with anti-CD40 and IL-4. Additional incubation with calcitriol resulted in a substantial reduction of NF-κB p50 and also p105 protein expression. In contrast, NF-κB p65 remained unchanged by anti-CD40 and IL-4 stimulation, but also additional calcitriol (Fig. 1A). To further elucidate whether the inhibition of p105/p50 protein expression was due to transcriptional downregulation of the precursor p105, qRT-PCR was performed with RNA isolated from naïve B cells. Stimulation with anti-CD40 and IL-4 increased p105 expression by 56 ± 5% (*p* = 0.008) compared to unstimulated B cells and calcitriol reduced p105 expression to 72 ± 5% (*p* = 0.003) of the stimulated control (Fig. 1B). These data show that VDR activation results in impaired NF-κB p105 expression. Our data are in line with previous findings that calcitriol reduces p105/p50 but not p65 protein levels in cytosol and nuclei of lymphocytes [17] and in nuclei of CD19<sup>+</sup> B cells [9]. Herein, we show for the first time that this inhibition of p105 expression is regulated at the transcriptional level by the activated VDR.

## 3.2. Reduced nuclear translocation of p65 upon vitamin D receptor activation

NF-κB p65 is one of the major transcription factors inducing NF-κB p105 transcription [18]. Although other transcription factors are involved as well, the importance of NF-κB is supported by the inhibition of PMA activation of the promoter by an NF-κB transdominant negative mutant [19]. We therefore analyzed the effect of calcitriol on p65 translocation. To avoid NF-κB activation during the induction of VDR in naïve B cells, the cells were initially stimulated with IL-4 alone for 24 h [16,20]. This procedure did not significantly activate NF-κB as shown by low nuclear p65 amounts (Fig. 1C). Accordingly, nuclear translocation of p65 was induced by stimulation with anti-CD40 for 1 h. The impact of VDR ligation was assessed by addition of calcitriol to the cells 1 h prior to CD40 activation. The data show that calcitriol considerably diminished p65 nuclear translocation (Fig. 1C). Thus, reduced p65 translocation

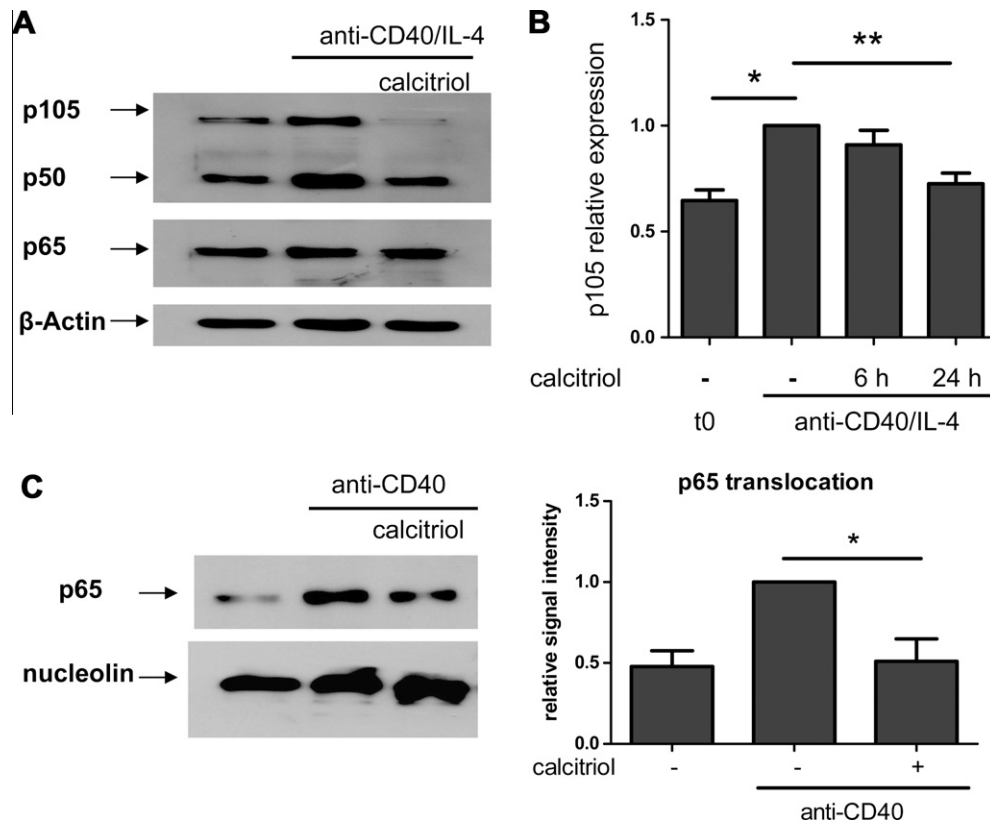


**Fig. 1.** VDR ligation inhibits NF- $\kappa$ B activation in human naive B cells. Naive B cells were magnetically sorted as CD19<sup>+</sup> CD27<sup>-</sup>. Cells were stimulated for 24 h with anti-CD40 and IL-4 in the presence or absence of 100 nM calcitriol. (A) Whole cell extracts were subjected to western blot analysis for NF- $\kappa$ B p105/p50 and NF- $\kappa$ B p65.  $\beta$ -Actin was used as a loading control. (B) p105 mRNA expression was determined in relation to HPRT.  $n \geq 8$ . \* $p \leq 0.05$ , \*\* $p \leq 0.005$ .  $t_0$ : ex vivo. (C) After 24 h stimulation with IL-4 alone nuclear translocation of p65 was induced with anti-CD40 for 1 h. Cells were prestimulated with calcitriol for 1 h and nuclear extracts were analyzed by western blot for p65. Nucleolin was used as a loading control ( $n = 5$ ). Left side: one representative experiment. Right side: mean and SEM of five independent experiments.

through activated VDRs may result in transcriptional downregulation of p105 after 24 h.

Generally, p65 is retained in the cytosol by the I $\kappa$ B-family. Among these I $\kappa$ B $\alpha$  has the highest affinity to p65 [21]. Upon stimulation with anti-CD40, I $\kappa$ B $\alpha$  is phosphorylated by the I $\kappa$ B kinase complex and subsequently degraded by the proteasome. Thereby, p65 can translocate to the nucleus. Herein, we analyzed whether I $\kappa$ B $\alpha$  is upregulated or whether its degradation is inhibited by calcitriol (Fig. 2). Our data show that in naive B cells I $\kappa$ B $\alpha$  degradation is stable upon calcitriol stimulation and thus, p65 translocation is reduced by an I $\kappa$ B $\alpha$  independent mechanism. However, the VDR impact on I $\kappa$ B $\alpha$  in different cell types is described heterogeneously, most likely due to cell specificity. VDR mediates increased I $\kappa$ B $\alpha$  expression in colon cancer cells [22], HL-60 leukemia cells [23], keratinocytes [24] and macrophages [4], whereas I $\kappa$ B $\alpha$  expression

is stable in human proximal tubular epithelial cells [25]. In fibroblast, calcitriol is reported to activate NF- $\kappa$ B by inducing I $\kappa$ B $\alpha$  phosphorylation [26].

### 3.3. Impaired binding of p65 to the p105 promoter by calcitriol

As NF- $\kappa$ B p65 is the main inducer of p105 transcription [18], we investigated whether the inhibition of p65 nuclear translocation leads to an impaired binding of p65 on the p105 promoter using ChIP. Naive B cells were stimulated as described for the p65 translocation analyses. Following fixation, the cell lysates were sonicated and p65 immunoprecipitation was performed. Binding of p65 protein to the p105 promoter was analyzed by qPCR-amplification of the promoter region containing a kappa-B ( $\kappa$ B) regulatory element. Amplification of the known p65 binding site in the I $\kappa$ B $\alpha$

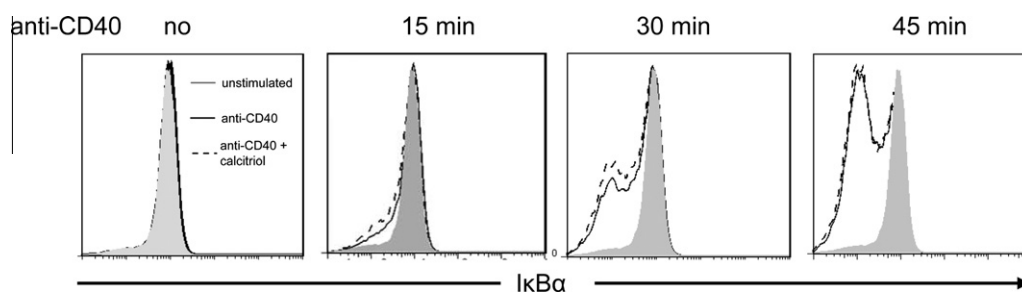


**Fig. 2.** I $\kappa$ B $\alpha$  degradation is unaffected by calcitriol treatment. B cells were stimulated with IL-4 for 24 h. I $\kappa$ B $\alpha$  degradation was induced by anti-CD40 stimulation for 15, 30 and 45 min, respectively. Cells were pretreated with 100 nM calcitriol for 1 h. Degradation of I $\kappa$ B $\alpha$  was assessed by flow cytometry, naive B cells were identified as CD19<sup>+</sup> CD27<sup>-</sup>. One representative experiment out of four is shown. Filled histograms: unstimulated cells, black lines: anti-CD40, dashed lines: anti-CD40 + calcitriol.

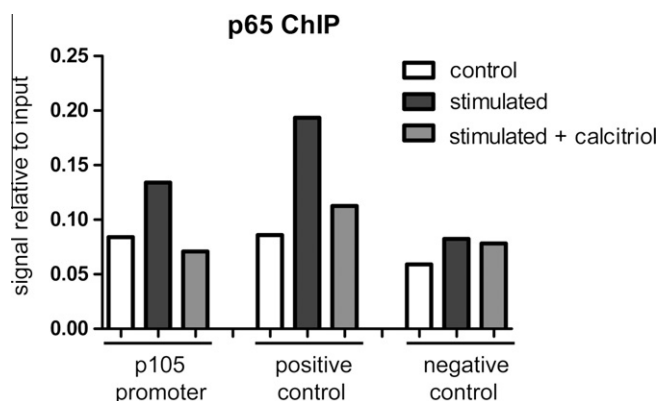


**Fig. 3.** VDR activation impairs binding of p65 to the p105 promoter. Naive B cells were stimulated with IL-4 for 24 h. p65 was activated with anti-CD40 with/out 1 h prestimulation with 1  $\mu$ M calcitriol and chromatin immunoprecipitation (ChIP) was performed with an anti-p65 antibody. p105 Promoter, I $\kappa$ B $\alpha$  promoter (positive control for p65 binding) and trpv6 ORF (negative control) were amplified by quantitative PCR. Signals are shown relative to input (unprecipitated) DNA. One representative experiment out of three is shown.

promoter proofed that p65 was activated and bound to DNA after anti-CD40 stimulation (Fig. 3). Anti-CD40 stimulation also resulted in p65 binding to the p105 promoter. Calcitriol stimulation reduced p65 binding to the p105 promoter back to baseline in unstimulated cells (Fig. 3). Accordingly, in the negative control region in the *trpv6* ORF that contains no p65 binding site, no activation or calcitriol-regulated p65 signal was obtained (Fig. 3).

The activation and differentiation of B cells to IgE secreting plasmablasts is inhibited by calcitriol *in vitro* and *in vivo*, most likely by impaired NF- $\kappa$ B p50 activation. Herein we show that VDR activation with calcitriol inhibits nuclear translocation of p65 by an I $\kappa$ B $\alpha$  independent mechanism. This results in reduced binding of p65 to the p105 promoter and subsequently in diminished expression of p105, thereby causing reduced protein levels of p105 as well as p50. Thus, VDR signaling may reduce or prevent activation of B cells and unwanted immune responses, e.g. in IgE dependent diseases such as allergic asthma.

## Acknowledgments

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB650-TP5) for KG, BH and GH. We thank Dennis Ernst for outstanding technical assistance.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bbrc.2011.03.078](https://doi.org/10.1016/j.bbrc.2011.03.078).

## References

- [1] T.-T. Wang, L.E. Tavera-Mendoza, D. Laperriere, E. Libby, N. Burton MacLeod, Y. Nagai, V. Bourdeau, A. Konstorium, B. Lallemand, R. Zhang, S. Mader, J.H. White, Large-scale in silico and microarray-based identification of direct 1, 25-dihydroxyvitamin D3 target genes, *Mol. Endocrinol.* 19 (2005) 2685–2695.
- [2] J.S. Adams, M. Hewison, Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity, *Nat. Clin. Pract. Endocrinol. Metab.* 4 (2008) 80–90.

- [3] J.R. Mora, M. Iwata, U.H. von Andrian, Vitamin effects on the immune system: vitamins A and D take centre stage, *Nat. Rev. Immunol.* (2008).
- [4] M. Cohen-Lahav, S. Shany, D. Tobvin, C. Chaimovitz, A. Douvdevani, Vitamin D decreases NF-kappaB activity by increasing IkappaBalpha levels, *Nephrol. Dial. Transplant.* 21 (2006) 889–897.
- [5] F.L. Szeto, J. Sun, J. Kong, Y. Duan, A. Liao, J.L. Madara, Y.C. Li, Involvement of the vitamin D receptor in the regulation of NF-kappaB activity in fibroblasts, *J. Steroid Biochem. Mol. Biol.* 103 (2007) 563–566.
- [6] A.A. Litonjua, S.T. Weiss, Is vitamin D deficiency to blame for the asthma epidemic?, *J. Allergy Clin. Immunol.* 120 (2007) 1031–1035.
- [7] E. Hyppönen, E. Läärä, A. Reunanen, M.-R. Järvelin, S.M. Virtanen, Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study, *Lancet* 358 (2001) 1500–1503.
- [8] A. Becker, R. Fischer, M. Schneider, Bone density and 25-OH vitamin D serum level in patients with systemic lupus erythematosus, *Zeitschrift für Rheumatologie* 60 (2001) 352–358.
- [9] G. Heine, K. Anton, B.M. Henz, M. Worm, 1Alpha, 25-dihydroxyvitamin D3 inhibits anti-CD40 plus IL-4-mediated IgE production in vitro, *Eur. J. Immunol.* 32 (2002) 3395–3404.
- [10] W.C. Sha, H.-C. Liou, E.I. Tuomanen, D. Baltimore, Targeted disruption of the p50 subunit of NF-[kappa]B leads to multifocal defects in immune responses, *Cell* 80 (1995) 321–330.
- [11] C.-L. Chen, N. Singh, F.E. Yull, D. Strayhorn, L. Van Kaer, L.D. Kerr, Lymphocytes lacking I(kappa)B-[alpha] develop normally, but have selective defects in proliferation and function, *J. Immunol.* 165 (2000) 5418–5427.
- [12] M. Milovanovic, G. Heine, W. Hallatschek, B. Opitz, A. Radbruch, M. Worm, Vitamin D receptor binds to the [epsilon] germ line gene promoter and exhibits transrepressive activity, *J. Allergy Clin. Immunol.* 126 (5) (2010) 1016–1023.
- [13] C. Weise, K. Hilt, M. Milovanovic, D. Ernst, R. Ruhl, M. Worm, Inhibition of IgE production by docosahexaenoic acid is mediated by direct interference with STAT6 and NFkappaB pathway in human B cells, *J. Nutr. Biochem.* (2010).
- [14] G. Heine, U. Niesner, H.D. Chang, A. Steinmeyer, U. Zugel, T. Zuberbier, A. Radbruch, M. Worm, 1, 25-Dihydroxyvitamin D(3) promotes IL-10 production in human B cells, *Eur. J. Immunol.* 38 (2008) 2210–2218.
- [15] U. Klein, K. Rajewsky, R. Kuppers, Human immunoglobulin (Ig)M + IgD + peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells, *J. Exp. Med.* 188 (1998) 1679–1689.
- [16] J.W. Morgan, N. Kouttab, D. Ford, A.L. Maizel, Vitamin D-mediated gene regulation in phenotypically defined human B cell subpopulations, *Endocrinology* 141 (2000) 3225–3234.
- [17] X.P. Yu, T. Bellido, S.C. Manolagas, Down-regulation of NF-kappa B protein levels in activated human lymphocytes by 1, 25-dihydroxyvitamin D3, *Proc. Natl. Acad. Sci. USA* 92 (1995) 10990–10994.
- [18] P.C. Cogswell, R.I. Scheinman, A.S. Baldwin Jr., Promoter of the human NF-kappa B p50/p105 gene. Regulation by NF-kappa B subunits and by c-REL, *J. Immunol.* 150 (1993) 2794–2804.
- [19] R.M. Ten, C.V. Paya, N. Israel, O. Le Bail, M.G. Mattei, J.L. Virelizier, P. Kourilsky, A. Israel, The characterization of the promoter of the gene encoding the p50 subunit of NF-kappa B indicates that it participates in its own regulation, *EMBO J.* 11 (1992) 195–203.
- [20] J.W. Morgan, D.J. Sliney, D.M. Morgan, A.L. Maizel, Differential regulation of gene transcription in subpopulations of human B lymphocytes by vitamin D3, *Endocrinology* 140 (1999) 381–391.
- [21] A. Oeckinghaus, S. Ghosh, The NF-kappaB family of transcription factors and its regulation, *Cold Spring Harbor Perspect. Biol.* 1 (2009) a000034.
- [22] J. Sun, R. Mustafi, S. Cerda, A. Chumsangri, Y.R. Xia, Y.C. Li, M. Bissonnette, Lithocholic acid down-regulation of NF-kappaB activity through vitamin D receptor in colonic cancer cells, *J. Steroid Biochem. Mol. Biol.* 111 (2008) 37–40.
- [23] A.K. Tse, C.K. Wan, X.L. Shen, G.Y. Zhu, H.Y. Cheung, M. Yang, W.F. Fong, 1, 25-Dihydroxyvitamin D3 induces biphasic NF-kappaB responses during HL-60 leukemia cells differentiation through protein induction and PI3K/Akt-dependent phosphorylation/degradation of IkappaB, *Exp. Cell Res.* 313 (2007) 1722–1734.
- [24] J.L. Riis, C. Johansen, B. Gesser, K. Møller, C.G. Larsen, K. Kragballe, L. Iversen, 1Alpha, 25(OH)(2)D(3) regulates NF-kappaB DNA binding activity in cultured normal human keratinocytes through an increase in IkappaBalpha expression, *Arch. Dermatol. Res.* 296 (2004) 195–202.
- [25] X. Tan, X. Wen, Y. Liu, Paricalcitol Inhibits Renal Inflammation by Promoting Vitamin D Receptor-Mediated Sequestration of NF-[kappa]B Signaling, *J. Am. Soc. Nephrol.* (2008).
- [26] L.S. Adams, D. Teegarden, 1, 25-Dihydroxycholecalciferol inhibits apoptosis in C3H10T1/2 murine fibroblast cells through activation of nuclear factor kappaB, *J. Nutr.* 134 (2004) 2948–2952.