

Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response

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Abstract

Background: 1 α ,25-dihydroxyvitamin D₃ (calcitriol), the biologically active form of vitamin D, is an immunomodulatory hormone, e.g. it inhibits IgE synthesis in B cells. As its clinical application is limited by hypercalcemia, synthetic vitamin D receptor (VDR) agonists that mediate immunomodulatory activities without adverse hypercalcemic effects are of great interest. This study aimed to investigate the impact of a low-calcemic VDR agonist on the IgE immune response *in vitro* and *in vivo*.

Methods: Human peripheral B cells were cultured under IgE inducing conditions in the presence of VDR ligands. B cells were analyzed by quantitative RT-PCR, enzyme-linked immunosorbent assays, enzyme-linked immunospot technique, and flow cytometry. BALB/c mice were sensitized with ovalbumin (OVA)/alum followed by the therapeutic VDR agonist treatment and analyzed regarding the humoral immunoglobulin profile.

Results: The natural VDR ligand calcitriol, but also a low-calcemic VDR agonist, profoundly suppressed IgE production by human peripheral B cells by $63.9 \pm 5.9\%$. The potential mechanisms involved are the reduction of the transcript for activation-induced deaminase (AID) and the reduction of IgE immunoglobulin-secreting cells by $68.1 \pm 12.7\%$. By using an *in vivo* approach, we finally demonstrate that the humoral IgE response in a type I allergy mouse model was impaired by the VDR agonist.

Conclusion: Our results show that targeting the VDR modulates the humoral immune response including IgE. Whether it might be useful for clinical applications has to be determined in appropriate clinical trials.

Immunoglobulin E (IgE) antibodies are the key effector molecules of type I allergic diseases. CD40L and IL-4 or IL-13 are the classical signals to induce class switch recombination to IgE in B cells by inducing the C ϵ germline transcript (ϵ GLT) (1). Another indispensable factor for class switching is the enzyme activation-induced cytidine deaminase (AID) (2). Upon activation by antigen-specific T_H2 cells, class-switched B cells further differentiate into long-lived memory B cells, into IgE-secreting plasmablasts and short-lived or resident long-lived plasma cells in the bone marrow (3).

Classical vitamin D functions are the regulation of calcium and phosphorous homeostasis and bone metabolism (4, 5). Additionally, immunomodulatory functions on several immune cell types have been described on the cellular and

molecular level (6, 7). Epidemiological data suggest that vitamin D insufficiency, reflected by the circulating precursor 25-hydroxyvitamin D₃ [25(OH)VD₃, < 30 ng/ml], is associated with elevated IgE levels (8). Other reports show a beneficial association between higher maternal vitamin D intake during pregnancy and protection from childhood asthma and allergic diseases in the offspring (9–11). In vitamin D receptor (VDR) knockout mice, IgE serum levels are increased (12). Previously, we have shown that calcitriol, the natural VDR agonist, directly inhibits the IgE production of stimulated human peripheral B lymphocytes *in vitro* (13).

Here, we analyzed the impact of a recently described low-calcemic VDR agonist (14) on the activation of different B cell subsets. Our results show that VDR activation inhibits IgE

and IgG, but also IgA to a lesser extent. Furthermore, we demonstrate that VDR signaling acts by at least two mechanisms, first by direct interference with the isotype switching machinery but also by a selective reduction of immunoglobulin-secreting cells (ISC). Finally, we proof our observations in a model of OVA-sensitized mice and show a decreased specific IgE response upon treatment with the low-calcemic agonist.

Material and methods

B cell purification

Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of healthy donors by density gradient separation (lymphocyte separation medium; PAA, Pasching, Austria), and B cells were purified by magnetic cell sorting using anti-CD19-coupled magnetic beads (purity $\geq 98\%$; Miltenyi Biotec, Bergisch-Gladbach, Germany). CD19⁺ CD27⁻ B cells were purified using an anti-CD19 MultiSort Kit followed by depletion of CD27⁺ and CD14⁺ cells (Miltenyi Biotec) or by FACS-sort using the FACSARIA Cell Sorter (BD Bioscience, San Jose, CA, USA). Sorted B cell populations were $>95\%$ enriched.

Cell culture

Purified peripheral human B cells (1×10^6 cells/ml) were cultured in Advanced RPMI 1640 culture medium (Invitrogen, Darmstadt, Germany) supplemented with 4 mM L-glutamine, 50 U/ml penicillin, 50 μ g/ml streptomycin, and 5% heat-inactivated charcoal stripped fetal calf serum (Biochrom, Berlin, Germany). Cells were stimulated with 1 μ g/ml anti-CD40 (Santa Cruz, Heidelberg, Germany), 10 ng/ml rhIL-4 (Miltenyi Biotec) with/out the synthetic VDR ligands ZK203278 (VDR agonist), ZK159222 (VDR antagonist; both provided by Bayer Schering Pharma AG, Berlin, Germany), or calcitriol (Sigma-Aldrich, Steinheim, Germany) at concentrations ranging from 1 to 100 nM. All cell cultures were carried out at 37°C and 5% CO₂ in a humidified atmosphere.

Flow cytometry

Cells were stained for 15 min at 4°C with directly conjugated mAb against human CD19 (Miltenyi Biotec), CD27, CD38, mouse CD21/CD35, CD23, CD19 (BD Pharmingen, San Diego, CA, USA). Stained cells were collected immediately using a FACSCalibur (Becton Dickinson, Heidelberg, Germany). Dead cells were excluded using 1 μ M propidium iodide (PI, Sigma-Aldrich). Data were analyzed using FLOWJO software (Tree Star, Inc., Ashland, OR, USA).

RNA extraction, cDNA synthesis, PCR, and quantitative PCR

For the analysis of *aicda*, *vdr* and *cyp24a1* expression, human CD19⁺ CD27⁻ and CD19⁺ CD27⁺ B cells were stimulated with anti-CD40 and IL-4 for 24 and 48 h with/out VDR ligands (100 nM). RNA extraction, reverse transcription of the RNA, and quantitative PCR were performed according to the manufacturer's protocols. All oligonucleotides were synthe-

sized by TIB MOLBIOL, Berlin, Germany; primer sequences for *hprt*, *vdr*, *cyp24a1*, and *aicda* were as described (15, 16). Relative quantification was performed as described in (16).

Human Ig-ELISA and Ig-ELISpot assay

Human B cells were cultured for 8 days, and immunoglobulin (Ig) concentrations were determined by ELISA, as described previously (16). Diluted human serum served as standard in which the respective Ig-levels were determined by the Charité clinical facility. The frequency of Ig-secreting cells [immunoglobulin-secreting cells (ISC)] was determined by ELISpot as described in (16).

Proliferation assay

The proliferation of activated B cells was monitored by 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution (Invitrogen) as described in (17). Briefly, cells were stimulated as above or additionally with 50 ng/ml IL-21 (ImmunoTools, Friesoythe, Germany) in the presence of VDR ligands for 5 days, and proliferation was assessed by flow cytometry excluding dead cells using 1 μ M PI.

Animal studies

Female, 10-week-old BALB/c mice (Federal Institute of Risk Assessment, Berlin, Germany) were kept in a pathogen-free environment under laboratory conditions. All experimental procedures were performed in compliance with protocols approved by the local State Office of Health and Social Affairs (LAGeSo). The mice were sensitized intraperitoneally using 10 μ g ovalbumin (OVA; Sigma-Aldrich) adsorbed to 1.5 mg aluminum hydroxide and magnesium hydroxide (Imject Alum; Thermo Fisher Scientific, Schwerte, Germany) as depicted in Fig. 6A. The VDR agonist (6 μ g/kg/day in PBS/0.03% ethanol) or an equal volume of PBS/0.03% ethanol as vehicle was injected intraperitoneally for two consecutive 5-day-periods separated by a 2-day-break (Fig. 6A). Sera and spleens were collected on day 47. Calcium serum concentrations were determined from pooled sera of each respective group on day 47 by the Charité clinical facility.

Murine Ig-ELISA

OVA-specific immunoglobulins were measured as described previously (17, 18). The concentrations of OVA-specific antibodies were estimated by comparison to a standard curve prepared from pooled sera of OVA-sensitized mice. Data are expressed as laboratory units per milliliter (LU/ml). For OVA-IgG₁, OVA-14 (Sigma-Aldrich) was used as standard. To analyze total murine IgE, MaxiSorp plates (Nunc, Wiesbaden, Germany) were coated with antimouse IgE (BD Pharmingen). After blocking of unspecific binding, murine IgE was detected with biotinylated anti-IgE (EM95.3, kindly provided by Dr Lamers, MPI, Freiburg, Germany) and streptavidin-conjugated horseradish peroxidase (R&D, Wiesbaden, Germany). The colorimetric reaction of peroxidase substrate

(TMB; Sigma-Aldrich) was measured at 450 nm after addition of 1 M sulfuric acid. Diluted mouse IgE κ (BD Pharmingen) was used for standard curve calculation.

Statistical analysis

Data were analyzed with PRISM 5.0 (GraphPad Software, La Jolla, CA, USA) using the paired *t*-test and nonparametric Wilcoxon signed rank test for matched pairs and Mann-Whitney *U*-test for independent groups. Data are shown as median or mean $\pm$ SEM. $P < 0.05$ was considered statistically significant.

Results

VDR activation inhibits IgE production

First, we assessed the expression of the VDR and the activity of the VDR agonist by investigating the effects on the expres-

sion of the vitamin D-responsive gene *cyp24a1* in human naïve and memory B cells as defined by their expression of CD19 and CD27 (19). *Cyp24a1*, a classical VDR-responsive gene (20), encodes the calcitriol-inactivating enzyme 25-OH-D₃-24-hydroxylase (21). In CD27[−] naïve B cells, the VDR agonist induced *cyp24a1* to a similar extent as calcitriol after 24 and 48 h; a slightly increased tendency of the *cyp24a1* induction in CD27⁺ memory vs CD27[−] naïve B cells was observed for both VDR ligands after 48 h (Fig. 1A). At the same time, *vdr* expression by itself, induced by anti-CD40/IL-4 stimulation, was not influenced by either the VDR agonist or by calcitriol (data not shown).

As we have previously shown that VDR targeting in B cells has an impact on immunoglobulin production, we studied the VDR agonist in comparison to calcitriol regarding IgE production. Indeed, we demonstrate that calcitriol but also the VDR agonist inhibits the anti-CD40/IL-4-induced IgE production in human B cells in a dose-dependent manner. A maximum of inhibition was reached with

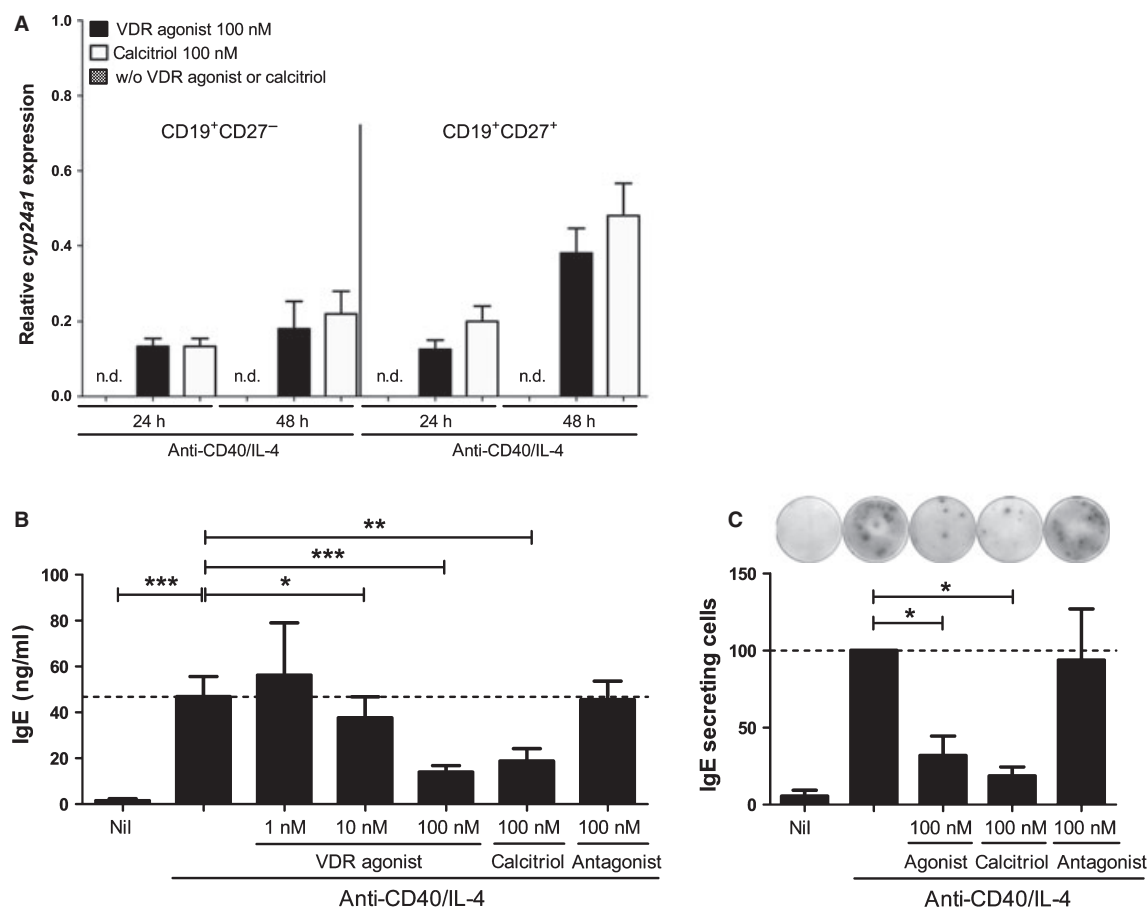


Figure 1 Vitamin D receptor (VDR) activation in human naïve and memory B cells and inhibition of IgE production by the VDR agonist. Human naïve and memory B cells were stimulated with anti-CD40 and IL-4 in the presence or absence of the VDR agonist or calcitriol for 24 and 48 h. (A) The mRNA of the vitamin D-responsive gene *cyp24a1* was assessed by quantitative PCR ($n \geq 4$, n.d. = not detectable). (B) B cells were activated in the presence of

various VDR ligands. IgE concentrations in the cell-free supernatants were determined by ELISA after 8 days of culture. The dashed line indicates the mean stimulated IgE concentration. Mean values $\pm$ SEM are given ($n \geq 3$). (C) B cells were cultured for 5 day as described in (B) and analyzed after 5 days by ELISpot assay. Data are shown as mean $\pm$ SEM in % of the anti-CD40/IL-4 stimulated control ($n = 6-7$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

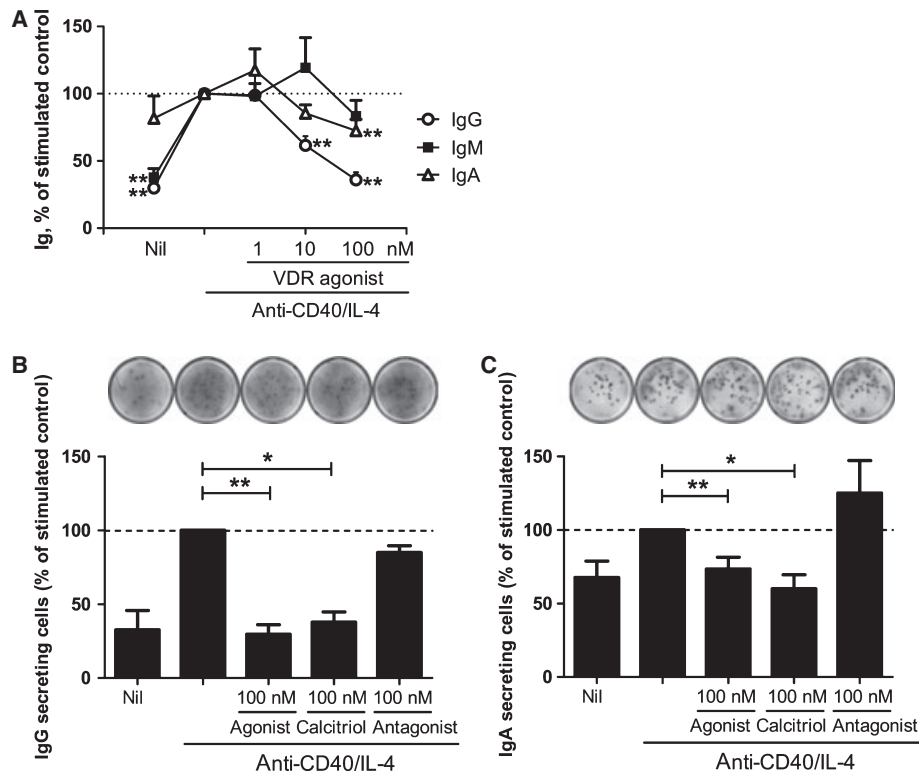


Figure 2 Analysis of other isotypes than IgE. (A) Peripheral CD19⁺ B cells were activated by anti-CD40 and IL-4 in the presence of increasing amounts of the vitamin D receptor (VDR) agonist. Ig concentrations in the cell-free supernatants were determined by ELISA after 8 days of culture ($n \geq 3$). Data are normalized to the anti-CD40/IL-4 stimulated control indicated by the dotted line. It corresponds to a mean concentration of IgG = 257.6 ± 94.4 ng/ml (○),

IgM = 629.4 ± 271.4 ng/ml (■) and IgA = 452.5 ± 98.4 ng/ml (△), respectively. (B and C) Peripheral CD19⁺ B cells were activated as described above in the presence or absence of 100 nM VDR agonist, calcitriol, or VDR antagonist. After 5 days of culture B cells were analyzed for IgG (B) and IgA (C) secreting B cells by ELISPOT assay. Data are shown as mean $\pm$ SEM in % of anti-CD40/IL-4 stimulated control ($n \geq 6$, * $P < 0.05$, ** $P < 0.01$).

$63.9 \pm 5.9\%$ (VDR agonist) and $64.8 \pm 10.6\%$ (calcitriol) (Fig. 1B). In order to address the question, if VDR signaling reduces the amount of secreted IgE per cell or inhibits differentiation to IgE-secreting cells, ELISPOT analysis was performed. Vitamin D receptor ligation by the agonist strongly reduced IgE-secreting cells by $68.1 \pm 12.7\%$ (Fig. 1C).

The secretion of IgG was inhibited upon VDR activation by the VDR agonist ($64.0 \pm 5.6\%$), whereas IgA was affected to a lesser extent ($27.6 \pm 8.5\%$) (Fig. 2A) which was also reflected by ELISPOT analyses (Fig. 2B, C). Conversely, IgM secretion was also reduced, but did not reach statistical significance (Fig. 2A).

Reduction of AID expression by calcitriol and the VDR agonist

We next investigated whether the inhibition of the switched isotypes by VDR targeting was due to changes in class switch recombination. Previously, we have shown that calcitriol inhibits the expression of ϵ GLT (13). The observation that IgG and IgA were significantly inhibited suggests common mechanisms that are also involved in IgE class switch recombination, namely expression of *aicda*.

The expression of *aicda* in CD19⁺ CD27[−] naïve B cells was inhibited by 33.2% after 24 h and by 39.6% after 48 h of culture (Fig. 3A). Calcitriol also reduced the expression of *aicda* in CD19⁺ CD27[−] B cells by 27.9% after 48 h (Fig. 3A). The *aicda* expression in CD19⁺ CD27⁺ memory B cells was inhibited by 29.1% after 24 h and by 35.7% after 48 h of incubation with the VDR agonist and to a comparable extent (28.1%) with calcitriol after 48 h (Fig. 3B). Together, both calcitriol and the low-calcemic VDR agonist interfere with the switching process by reducing *aicda* expression.

Reduction of the CD19⁺ CD27^{high} CD38⁺ B cell population by activated VDRs

Having found a suppressive effect on AID, but even more potentially, on the production of distinctive Ig subclasses themselves, we asked whether additional events may also be targets of VDR-dependent action.

Therefore, B cell proliferation was analyzed by CFSE dilution assays. As shown in Fig. 4A, little proliferation of B cells upon anti-CD40/IL-4 stimulation was observed. The VDR agonist resulted in a slightly reduced cell frequency of proliferating cells to $10.8 \pm 1.0\%$ compared

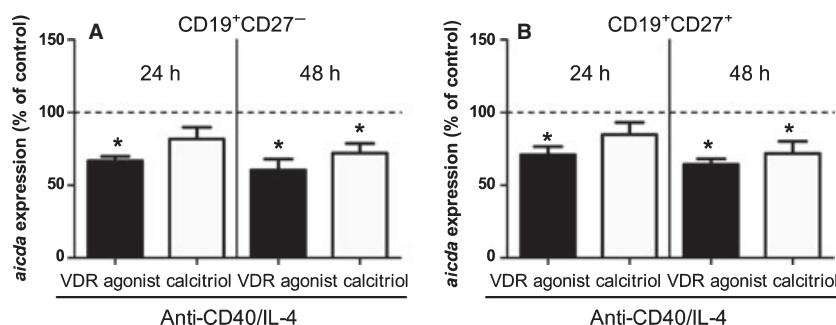


Figure 3 Inhibition of *aicda* expression. (A) Human naïve B cells (CD19⁺ CD27⁻) were stimulated with anti-CD40 and IL-4 in the presence or absence of the vitamin D receptor agonist or calcitriol for 24 and 48 h. The mRNA of the activation-induced cytidine deaminase (*aicda*) was assessed by quantitative PCR. (B) Memory

B cells (CD19⁺ CD27⁺) were analyzed as described in (A). The relative quantification was calculated and is given in % of the anti-CD40/IL-4 stimulated control. The percentages are shown as mean ± SEM ($n \geq 6$, * $P < 0.05$).

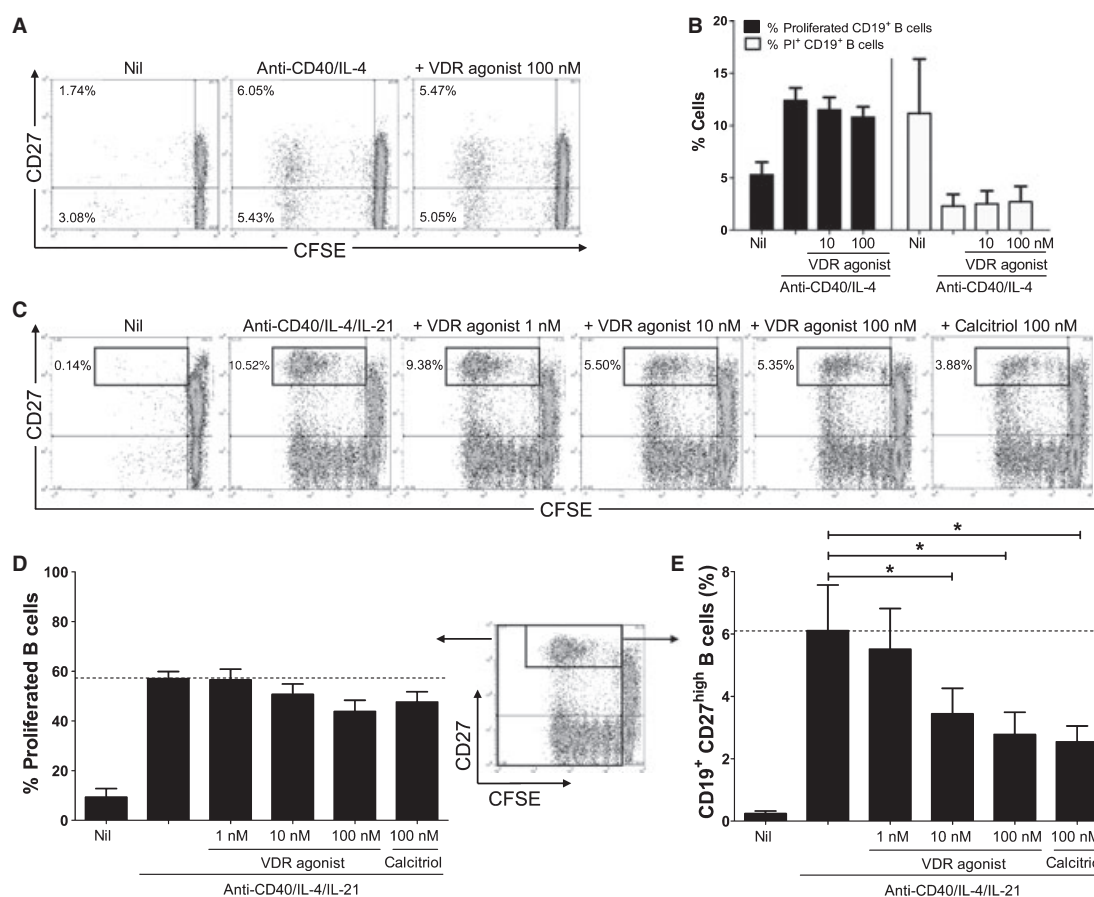


Figure 4 B cell proliferation and generation of CD27^{high} cells. (A and B) Cells were labeled with CFSE and stimulated with anti-CD40 and IL-4 in the presence or absence of 100 nM vitamin D receptor agonist. After a 5-day-culture, cells were analyzed by flow cytometry and the frequency of proliferated and PI⁺ cells was determined. The percentages of proliferated and PI-positive cells were measured and

are shown as mean ± SEM ($n = 4$). (C–E) Peripheral human CD19⁺ B cells were cultured as described earlier with the addition of IL-21 and analyzed after 5 days. (C) Representative density plots. (D) The percentages of proliferated CD19⁺ B cells are shown as mean ± SEM ($n = 4$). (E) The frequency of CD19⁺ CD27^{high} B cells is shown as mean ± SEM ($n = 7$; * $P < 0.05$).

with $12.4 \pm 1.2\%$ ($P > 0.05$), while the percentages of PI-positive cells remained stable (Fig. 4B). Next, we assessed whether B cell proliferation may be more strongly affected

under highly proliferative conditions through simultaneous treatment with IL-21 and anti-CD40/IL-4. As expected, B cell proliferation was 4.6-fold more pronounced under these con-

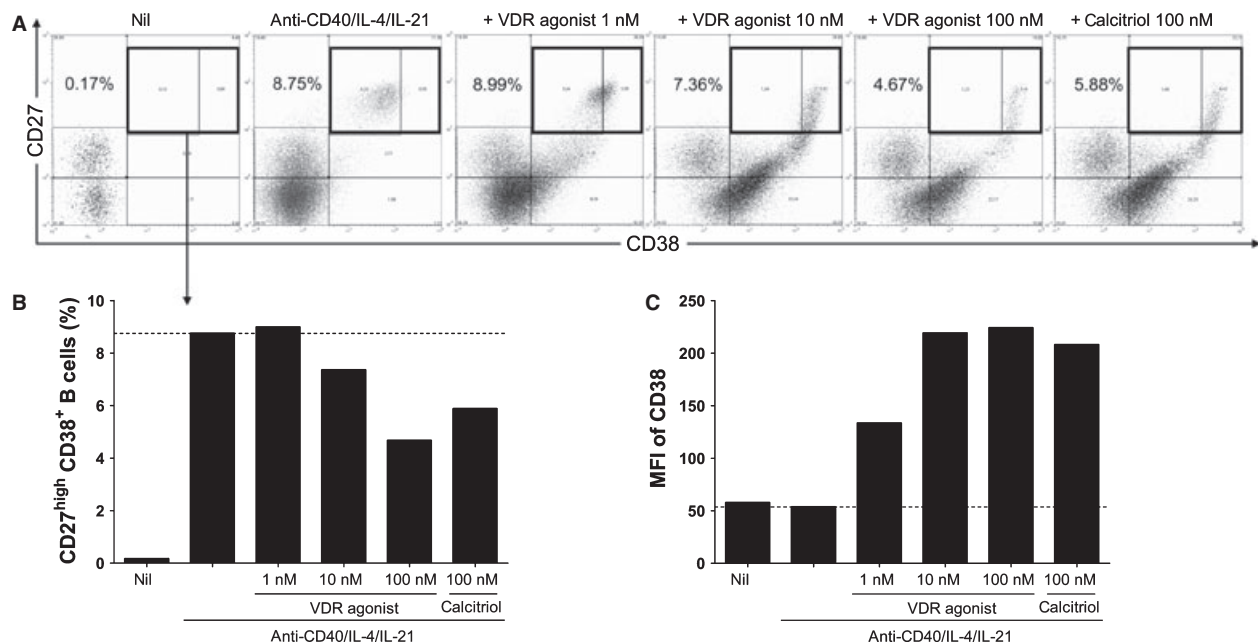


Figure 5 Vitamin D receptor (VDR) targeting reduces the frequency of CD27^{high} CD38⁺ B cells while increasing the expression of CD38. (A) Human peripheral CD19⁺ B cells were stimulated with anti-CD40/IL-4 and IL-21 in the presence or absence of increasing amounts of the VDR agonist and calcitriol. After a 5-day-culture,

cells were analyzed by flow cytometry. (B) Representative percentages of CD19⁺, PI⁻, CD27^{high}, CD38⁺ B cells are shown. (C) The MFI of CD38, gated on CD19⁺, PI⁻, CD27^{high} cells, was determined. Shown is one representative out of three experiments. MFI, median fluorescence intensity.

ditions as determined by increased CFSE dilution corresponding to a higher number of proliferating cells (Fig. 4C). Notably, in B cells treated with the VDR agonist, a slight, but not significant antiproliferative effect of $16.6 \pm 7.4\%$ (100 nM) was observed under these conditions (Fig. 4D).

Additionally, IL-21 is well known to facilitate the differentiation of human B cells into ISC that are characterized as CD27^{high} and CD38⁺ (22, 23). In our study, $6.1 \pm 1.5\%$ of ISC were induced by anti-CD40/IL-4 and IL-21. Upon addition of the VDR agonist, this population was diminished dose-dependently, with a maximum inhibition of $51.1 \pm 6.3\%$ (Figs 4C, E and 5A, B). Expression of the VDR target gene CD38 (15, 24) on these cells was increased indicating recent VDR activation (Fig. 5C).

VDR activation reduces the IgE response in a type I allergy mouse model without calcemic side-effects

Based on the potency to counteract the IgE production *in vitro*, we were interested to delineate the activity of the low-calcemic VDR agonist on the allergic immune response *in vivo*. Mice were sensitized and treated as depicted in Fig. 6A. The low-calcemic nature of the VDR agonist was confirmed by measurement of serum calcium concentrations on day 47 (VDR agonist treated group: 2.37 mM; PBS treated group: 2.36 mM) (14) (Fig. 6B). In addition, the mice remained healthy and displayed comparable development of weight gain throughout the experiment (Fig. 6C). Our data show that the therapeutic treatment regimen led to a pro-

found inhibition of the allergen-specific IgE response by 46.7% ($P = 0.03$, Fig. 6D, right). As expected, no allergen-specific Ig production could be measured in nonsensitized control mice (data not shown). Total IgE levels were also reduced by 35.5% ($P = 0.05$, Fig. 6D, left). Although the effect on the IgE level was most distinctive, we also observed an inhibition of the allergen-specific IgG₁ levels by 20.7% ($P = 0.04$, Fig. 6E). A similar tendency as for IgG₁ was found for IgG_{2a} and IgA, but overall levels of these isotypes were barely detectable and variable (data not shown). To assess whether the suppressing effects on IgE production were accompanied by alterations in the B cell compartment, we assessed the composition of splenic B cells. As is shown in Fig. 6F, the B cell compartment consisted of $5.3 \pm 0.2\%$ marginal zone B cells, expressing high levels of CD21 but no CD23, and $80.9 \pm 0.9\%$ of follicular B cells, expressing CD21 and CD23. This pattern did not change upon VDR activation, demonstrating that the suppression of IgE production is not the result of overt changes in B cell subpopulations, further emphasizing the specificity of the Ig reducing effect.

Discussion

Herein, we show in a proof of concept experiment that activating VDRs by a low-calcemic agonist results in modulation of the humoral IgE response.

Calcitriol mediates anti-inflammatory functions on adaptive immune responses including IgE synthesis (6, 7, 13), but

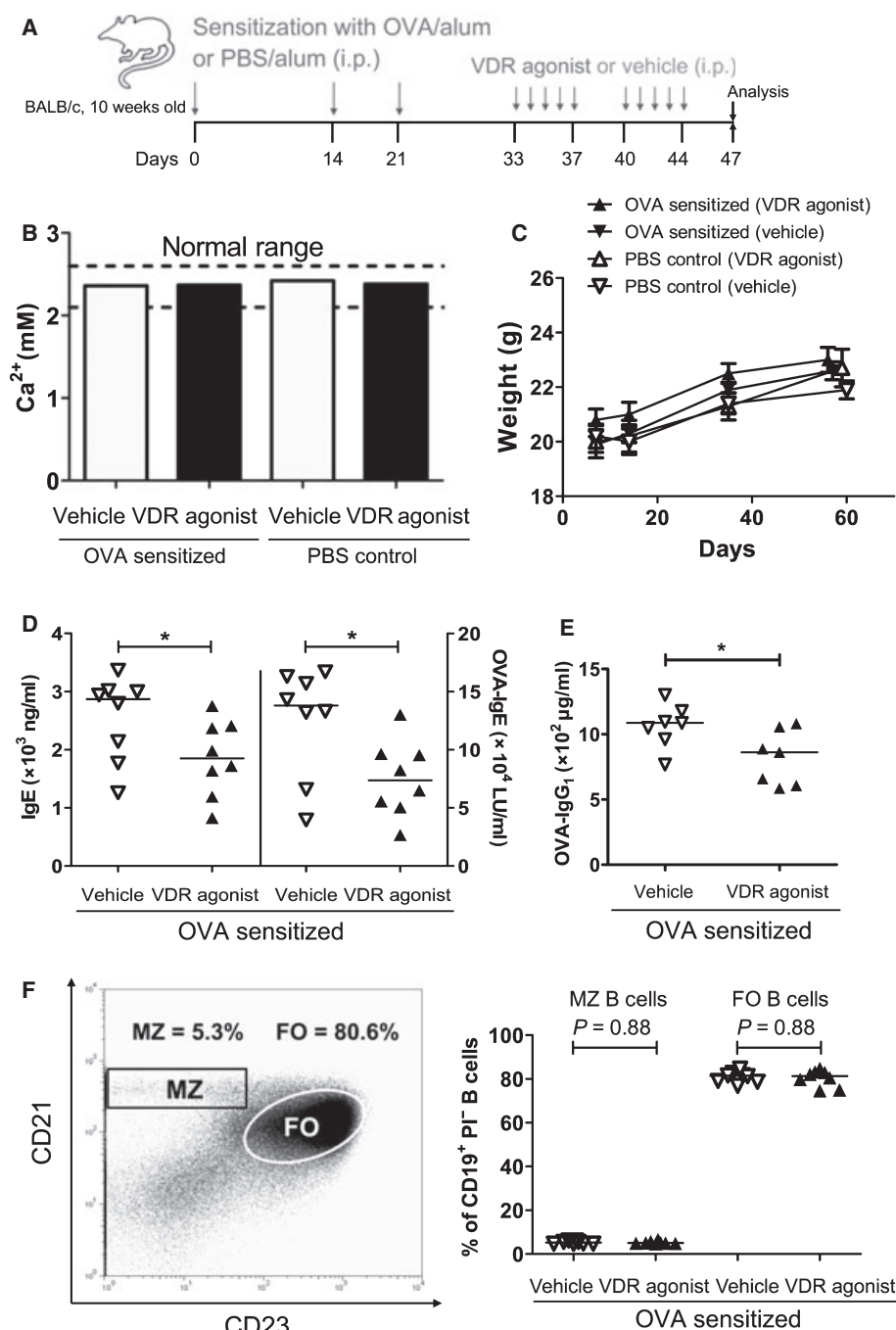


Figure 6 Reduction of total and allergen-specific IgE *in vivo*. (A) Ten-week-old female BALB/c mice were sensitized three times intraperitoneally with OVA/alum on days 0, 14, and 21. Mice were treated by intraperitoneal injections with either the vitamin D receptor (VDR) agonist or PBS (0.03% ethanol) as described in materials and methods. (B) Ca^{2+} levels in pooled sera from sensitized and nonsensitized BALB/c mice on day 47, treated with the VDR agonist or vehicle. (C) Weight development over time of VDR agonist treated mice compared to vehicle treated control groups. Data are

the means $\pm$ SEM of eight mice per group. (D and E) Total IgE (D, left), allergen-specific IgE (D, right), and IgG₁ levels (E) in sera of treated mice on day 47 were determined by ELISA. (F) The splenic B cell compartment gated on live CD19⁺ B cells was analyzed by flow cytometry. Follicular (FO, CD21^{mid}, CD23⁺) and marginal zone (MZ, CD21^{hi}, CD23⁻) B cells were identified by different expression levels of CD21 and CD23. Bars in the scatter plot represent medians ($n \geq 7$), triangles represent single values (* $P < 0.05$).

its use is limited by pronounced hypercalcemic side-effects in pharmacological dosages resulting in significant toxicity when systemically applied (25–29). Thus, synthetic analogs with reduced hypercalcemic activity were developed for systemic application. The VDR agonist used in the present study recently showed immunomodulatory properties *in vitro* and *in vivo* (14, 30). The advantage of the VDR agonist compared with calcitriol is its dissociated profile (immunomodulatory *vs* calcemic). Thus, the VDR agonist being immunomodulatory while not being hypercalcemic suggests the existence of a broad therapeutic safety window (14).

Our data show that the VDR agonist activates the VDR in naïve and memory human B cells, reflected by *cyp24a1* gene expression. We demonstrate that the VDR agonist inhibits the anti-CD40/IL-4-induced IgE production that is associated with a reduced number of IgE-secreting B cells. At the molecular level, we identified an inhibition of *aicda* expression, encoding AID, which is indispensable for somatic hypermutation and class switching toward IgE but also other subtypes in both subsets (1, 2). The diminished expression of *aicda* may contribute to the decreased immunoglobulin production of all isotypes other than IgM and IgD, as it is required for class switch recombination.

As B cell proliferation is associated with isotype class switching as well as immunoglobulin secretion (31), we investigated the impact of VDR activation on proliferation in more detail. We observed that proliferation of anti-CD40/IL-4-activated human B cells with or without additional mitogenic IL-21 is not significantly altered by the VDR agonist. The early B cell activation, as defined by the surface markers CD23 (FcεRII) and CD69, was not affected in the initial phase of culture (data not shown). Nevertheless, the slight antiproliferative effect of the VDR agonist may contribute to the observed reduction in Ig production. In fact, a strong link between differentiation of human B cells to IgE-secreting cells and the degree of cell division is well documented (32). This antiproliferative effect can, however, not fully explain the remarkable inhibition of IgE secretion by $63.9 \pm 5.9\%$ and the reduction of other isotypes like IgG and IgA.

Because of the strong reduction in the number of ISC, we analyzed the differentiation of CD27^{high} CD38⁺ B cells which define *in vitro* generated ISC that express a transcriptional profile comparable to plasma cells (23, 33). Indeed, diminished frequencies of CD27^{high} CD38⁺ B cells were detected, although CD38 expression, a well known target of VDR activation (15, 24), was increased on the CD27^{high} subset. The clear reduction of this cell population may well contribute to the reduced number of Ig-secreting cells correlating with the reduced Ig secretion of activated B cells. Such reduc-

tion of plasma blasts is in accordance with previous data using calcitriol (34). Taken together, we identified two potential mechanisms by which the VDR agonist may inhibit the IgE response, first by interference with the switching machinery and secondly by reducing the differentiation of Ig-secreting cells. Unravelling the mechanistic details in their entirety and determination of the relative contribution of the different pathways modified upon VDR activation will require future experiments.

Based on these *in vitro* observations, we investigated the immune modulation by the VDR agonist in a murine model of type I sensitization. As the serum calcium concentration in treated groups remained stable, hypercalcemic effects were excluded, which clearly supports the advantage of this VDR agonist in pharmacological doses in line with previous findings (14). Importantly, a strong reduction of the allergen-specific IgE response and to a lesser degree total serum IgE production was observed. We also determined a reduction of the allergen-specific IgG₁ response, although the extent of suppression was clearly more pronounced on specific IgE than IgG₁. There are only a few reports that directly addressed the role of VDR signaling for allergic immune responses *in vivo*, but these support the notion that VDR triggering possesses anti-allergic effects. Accordingly, type I sensitized VDR knockout mice have higher serum IgE concentrations than wild-type controls in an allergic asthma model (12), suggesting an important role for VDR signaling in regulating IgE responses *in vivo*. Additionally, allergen-specific immunotherapy has been suggested to be more efficient upon calcitriol treatment (35). However, so far no data are available on such a concept in human allergic patients.

In conclusion, our data provide evidence that VDR activation by a low-calcemic agonist modulates the humoral immune response including IgE. Thus, targeting VDRs in B cells by a low-calcemic ligand may be a promising strategy for novel therapeutic approaches.

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Conflict of interest

The authors declare no conflict of interest. The authors (U. Zügel, A. Steinmeyer) are employees of Bayer Schering Pharma AG and are involved in drug finding programs.

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