1α,25-Dihydroxyvitamin D_3 decreases DNA binding of nuclear factor-κB in human fibroblasts

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Abstract 1α,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2$-D_3), the active metabolite of vitamin D, can inhibit NF-κB activity in human MRC-5 fibroblasts, targeting DNA binding of NF-κB but not translocation of its subunits p50 and p65. The partial inhibition of NF-κB DNA binding by 1,25-$(OH)_2$-D_3 is dependent on de novo protein synthesis, suggesting that 1,25-$(OH)_2$-D_3 may regulate expression of cellular factors which contribute to reduced DNA binding of NF-κB. Although NF-κB binding is decreased by 1,25-$(OH)_2$-D_3 in MRC-5 cells, IL-8 and IL-6 mRNA levels are only moderately downregulated, demonstrating that inhibition of NF-κB DNA binding alone is not sufficient for optimal downregulation of these genes.

Key words: 1α,25-Dihydroxyvitamin D_3; Fibroblast; Interleukin-8; Nuclear factor-κB; Tumor necrosis factor-α

1. Introduction

1α,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2$-D_3; calcitriol), the hormonally active form of vitamin D, is a potent regulator of calcium and skeletal homeostasis which also exerts other activities, including induction of differentiation and inhibition of proliferation in various cell systems [1]. 1,25-$(OH)_2$-D_3 exerts its effects by binding to its nuclear receptor, the vitamin D receptor (VDR), a member of the superfamily of nuclear receptors [2]. Like several of these receptors, VDR has been shown to bind as a heterodimer with the retinoid X receptor (RXR) to DNA sequences called vitamin D response elements (VDRE) [3–6].

1,25-$(OH)_2$-D_3 also has immunomodulatory properties [7] and can downregulate the expression of some cytokines. At the molecular level, VDR has been shown to inhibit the activity of the transcription factor complex AP-1 on the osteocalcin promoter [8]. 1,25-$(OH)_2$-D_3 has also been shown to repress IL-2 gene expression in T lymphocytes by inhibiting complex formation between nuclear factor of activated T cells (NFATp) and AP-1 at the NFAT binding site of the IL-2 promoter. In vitro analysis has shown that VDR can bind as a VDR/RXR heterodimer to the NFAT binding site of the IL-2 promoter [9,10]. Another transcription factor which can be affected by 1,25-$(OH)_2$-D_3 is nuclear factor-κB (NF-κB). NF-κB is an inducible transcription factor, representing a family of related proteins including p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel and RelB. NF-κB plays an essential role in the transcriptional regulation of inflammatory genes, such as IL-8, IL-6, ICAM-1, E-selectin and IFN-β [11–14]. NF-κB is bound in the cytosol to inhibitor proteins, members of the IκB family. Phosphorylation of IκB after cell activation is associated with release of NF-κB which then translocates to the nucleus [15–19].

1,25-$(OH)_2$-D_3 has been reported to downregulate the levels of the NF-κB protein p50 and its precursor p105 in human lymphocytes [20]. We have shown in a previous report that TNF-α-induced IL-8 gene expression is repressed in a human melanoma cell line by 1,25-$(OH)_2$-D_3 at the transcriptional level, involving the NF-κB binding site of the IL-8 promoter [21]. NF-κB is further involved in the downregulation of IL-12 expression by 1,25-$(OH)_2$-D_3 in human monocytes and dendritic cells [22]. In addition to its classical therapeutic effects, 1,25-$(OH)_2$-D_3 may therefore be of interest as an anti-inflammatory principle. Since fibroblasts, which can play a major role in inflammatory processes, have readily inducible NF-κB activity and express high levels of IL-8, we investigated whether NF-κB is a target of 1,25-$(OH)_2$-D_3 in this cell type. In contrast to the glucocorticoid dexamethasone, 1,25-$(OH)_2$-D_3 caused a partial inhibition of cytokine-induced NF-κB DNA binding in these cells, an effect which appears not mediated by VDR directly, but rather via other cellular, 1,25-$(OH)_2$-D_3-inducible factors.

2. Materials and methods

2.1. Materials

1,25-$(OH)_2$-D_3 was obtained from Calbiochem (La Jolla, CA). Dexamethasone was purchased from Sigma (St. Louis, MO) and stored as a 1 mM stock in methanol at −20°C. Recombinant human TNF-α (2×10^7 U/mg) was purchased from Genzyme (Cambridge, MA). Recombinant human IL-1β was from P. Ramage (Novartis Pharma, Basel).

2.2. Cell culture

Normal human embryonic lung fibroblasts MRC-5 cells were purchased from ATCC (Rockville, MD). Cells were cultivated in MEM supplemented with 10% FCS in an humidified 5% CO_2 atmosphere, and were kept for no longer than 15 passages.

2.3. Immunofluorescence

7×10^4 cells were seeded into each well of eight-well LabTek slides (Nunc, Naperville, IL) and treated as described in the text. Cells were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.5% Triton X-100 for 5 min, and then incubated for 1 h with a rabbit polyclonal antibody against NF-κB p65 or a goat polyclonal antibody against NF-κB p50 (both from Santa Cruz Biotechnology, Santa Cruz, CA) at room temperature. Detection was carried out using a rhodamine-conjugated goat anti-rabbit antibody (Accurate Scientific, Westbury, NY) or a fluorescein isothiocyanate-coupled anti-goat IgG (Santa Cruz Biotechnology). The slides were examined with a Bio-

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Abbreviations: 1,25-$(OH)_2$-D_3, 1α,25-dihydroxyvitamin D_3; CHX, cycloheximide; EMSA, electrophoretic mobility shift assay; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α

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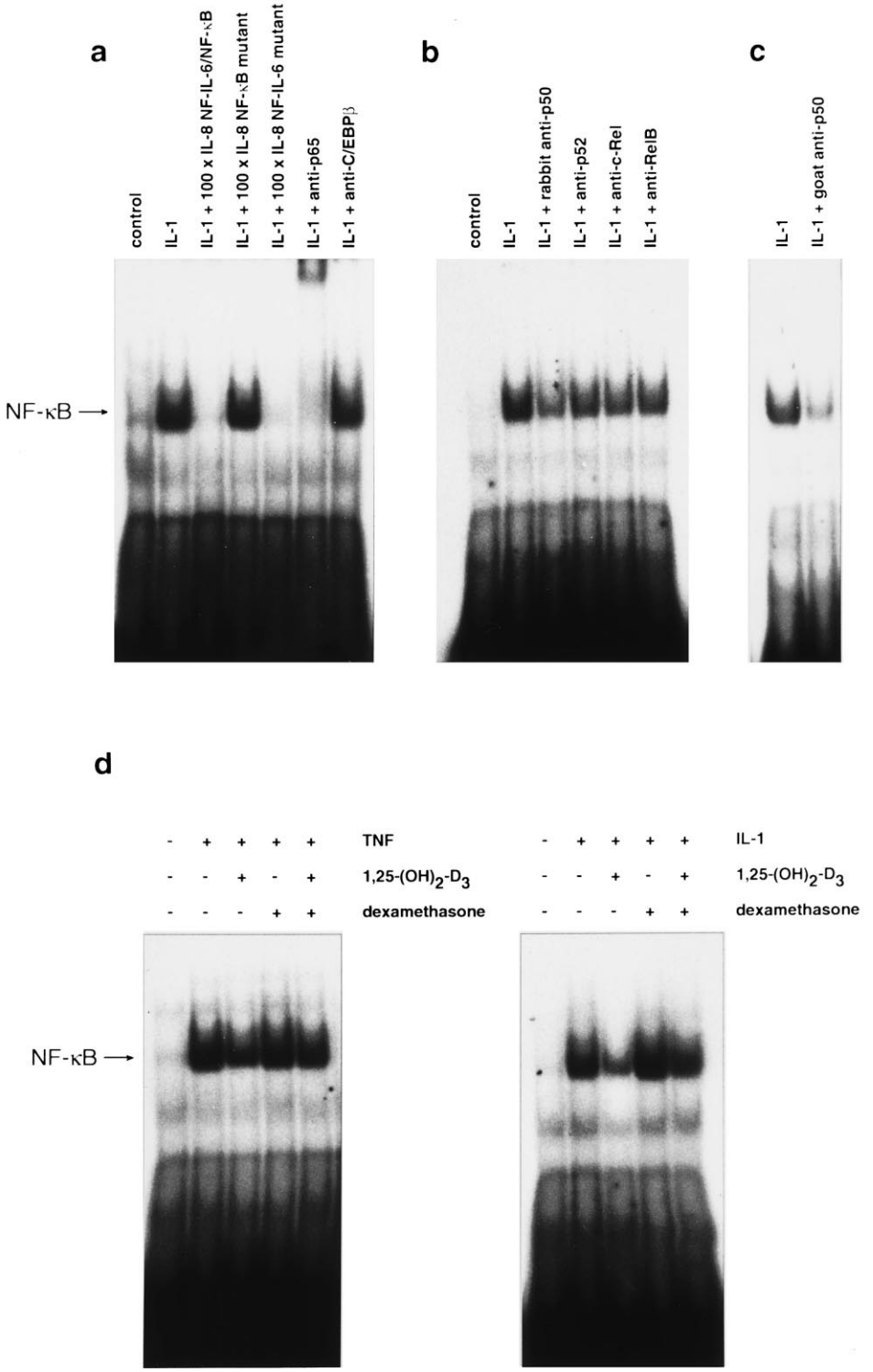

Fig. 1. Binding of NF-κB to its binding site in the IL-8 promoter is decreased by 1,25-$(OH)_2$-D_3 but not dexamethasone. a: 2×10^6 cells were treated with IL-1β (50 ng/ml) for 1 h before preparation of nuclear extracts. For EMSA, about 5 μg of the extracts were incubated with 0.4 ng radiolabeled oligonucleotide (IL-8 wt NF-IL-6/NF-κB). 100-fold molar excess of unlabeled oligonucleotides was used for competition. For supershift analysis, 2 μl of anti-p65 or anti-C/EBPβ antibody was added to the binding reaction and kept at room temperature for 20 min prior to addition of poly dI.dC and the radiolabeled IL-8 wt NF-IL-6/NF-κB oligonucleotide. b: Nuclear extracts from IL-1β-stimulated MRC-5 cells were incubated with 2 μl of anti-p50 (rabbit), 2 μl anti-p52, 2 μl anti-c-Rel or 2 μl anti-RelB antibody for 20 min at room temperature prior to addition of poly dI.dC and radiolabeled IL-8 wt NF-IL-6/NF-κB oligonucleotide. c: The same extract was incubated with 2 μl of anti-p50 (goat) antibody, comfirming the presence of p50 in the complex. d: 2×10^6 cells were treated with 1,25-$(OH)_2$-D_3 (20 nM), dexamethasone (1 μM) or both for 23 h before stimulation with either TNF-α (200 U/ml) or IL-1β (50 ng/ml) for 1 h. Controls were treated with vehicle only (0.1% ethanol), TNF-α or IL-1β alone. EMSAs were performed with nuclear extracts incubated with the IL-8 wt NF-IL-6/NF-κB radiolabeled oligonucleotide. The decrease in NF-κB DNA binding by 1,25-$(OH)_2$-D_3 was observed in at least five individual preparations.

Rad MRC 600 confocal laser scanning microscope (Bio-Rad, Hemel Hempstead, UK), mounted on a Zeiss IM 10 microscope (Zeiss, Vienna, Austria).

2.4. Electrophoretic mobility shift assays

For electrophoretic mobility shift assay (EMSA) experiments the following oligonucleotides were used: IL-8 wt NF-IL-6/NF-κB: 5′-AATTCCAGTTGCAAATCGTGGAATTTCCTG-3′, IL-8 NF-κB mutant: 5′-AATTCCAGTTGCAAATCGTGGCCGGTCCTG-3′; IL-8 NF-IL-6 mutant: 5′-AATTCCAGCTACGAGTCGTGGAATTTCCTG-3′; p50 NF-κB: 5′-GATCTCGACGTCAGTGGGAATTTCCAG-3′; IL-6 NF-κB: 5′-GATCTATGTGGGATTTTCCCATG-3′; HIV LTR NF-κB: 5′-GATCTTGTTACAAGGGACTTTCCGCG-3′. Nuclear extracts and EMSA experiments were performed as described previously [23]. All antibodies for supershift experiments were from Santa Cruz (Santa Cruz, CA).

2.5. Isolation of RNA and Northern analysis

MRC-5 cells were seeded at a density of 5×10^5 cells per well of a six-well plate and allowed to attach overnight. On the next day, cells were treated as described in the text. Total RNA was prepared using the Ultraspec RNA isolation reagent (Biotecx Laboratories, Houston, TX). Northern blot analyses were performed as previously described [23]. IL-8 was detected using a 350 bp fragment comprising the coding region of the IL-8 cDNA. IL-6 cDNA was kindly provided by R. Movva (Novartis Pharma, Basel). Glyceraldehyde 3-phosphate dehydrogenase was detected using a *Eco*RI-*Hin*dIII fragment of the rat cDNA, kindly provided by P. Amstad (ISREC, Lausanne).

3. Results

3.1. Binding of NF-κB to its binding site in the IL-8 promoter is decreased by 1,25-$(OH)_2$-D_3 but not dexamethasone in MRC-5 human fibroblasts

EMSA using nuclear extracts from MRC-5 cells stimulated with IL-1β for 1 h revealed a single complex with a labeled oligonucleotide representing the nucleotides −94 to −71 relative to the transcription start of the IL-8 gene, containing the nuclear factor for interleukin-6 (NF-IL-6) and NF-κB binding sites of the IL-8 promoter (IL-8 wt NF-IL-6/NF-κB). Competition experiments, using 100-fold molar excess of unlabeled oligonucleotides comprising mutated sequences (IL-8 NF-κB mutant, IL-8 NF-IL-6 mutant) and supershift experiments using antibodies directed against the NF-κB subunit p65 and CAAT/enhancer binding protein β (C/EBPβ/NF-IL-6), demonstrated that this binding occurs specifically on the NF-κB binding site of the IL-8 promoter and does not involve C/EBPβ/NF-IL-6 (Fig. 1a). To determine whether the complex contains a p65 homodimer or whether other NF-κB proteins are involved, supershift experiments were performed using antibodies directed against the NF-κB proteins p50, p52, c-Rel and RelB. Although the antibody against p50 did not apparently shift the complex, binding to the radiolabeled oligonucleotide was reduced (Fig. 1b). Similar results were obtained with a different anti-p50 antibody (Fig. 1c), indicating that the observed complex contains a p50/p65 heterodimer.

Stimulation with TNF-α for 1 h also induced NF-κB complex formation in MRC-5 cells, but preincubation of cells with 1,25-$(OH)_2$-D_3 for 23 h reduced this binding. Treatment of cells with 1,25-$(OH)_2$-D_3 for 23 h followed by a 1 h stimulation with IL-1β resulted in a significant reduction of NF-κB binding compared to that induced by IL-1β alone. In contrast, pretreatment of cells with 1 μM dexamethasone for 23 h preceding a 1 h stimulation with either IL-1β or TNF-α did not reduce NF-κB binding to the IL-8 promoter sequence. There was even an enhanced binding of the IL-1β-induced NF-κB complex observed when cells were prestimulated with dexamethasone, which also partially restored the reduced NF-κB binding by 1,25-$(OH)_2$-D_3 (Fig. 1d). These results indicate that 1,25-$(OH)_2$-D_3 has an activity on NF-κB which is distinct from that of dexamethasone.

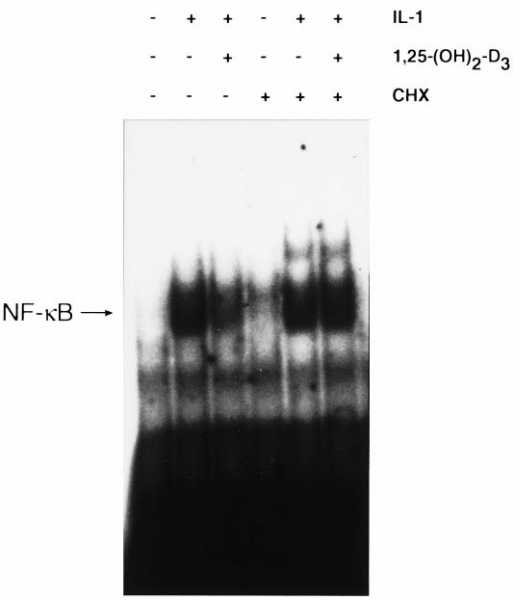

Fig. 2. Reduction of NF-κB binding requires de novo protein synthesis. 2×10^6 cells were treated with 1,25-$(OH)_2$-D_3 (20 nM) for 23 h preceding a 1 h stimulation with IL-1β (50 ng/ml) or treated simultaneously with cycloheximide (CHX; 5 μg/ml) and 1,25-$(OH)_2$-D_3 (20 nM) for 23 h before stimulation with IL-1β for 1 h. Controls were treated with vehicle (0.1% ethanol), for 23 h with CHX plus vehicle or CHX with an additional stimulation for 1 h with IL-1β. EMSAs were performed with nuclear extracts incubated with the IL-8 wt NF-IL-6/NF-κB radiolabeled oligonucleotide.

3.2. Reduction of NF-κB binding requires de novo protein synthesis

Cells were treated with the protein synthesis inhibitor cycloheximide (CHX; 5 μg/ml) alone or simultaneously with 1,25-$(OH)_2$-D_3 before IL-1β stimulation for 1 h. EMSA studies showed that 23 h pretreatment with CHX had no effect on IL-1β-induced binding of NF-κB to the oligonucleotide (IL-8 wt NF-IL-6/NF-κB). However, when cells were simultaneously exposed to CHX and 1,25-$(OH)_2$-D_3, no reduction in IL-1β-induced NF-κB complex formation was seen. This CHX-mediated inhibition of 1,25-$(OH)_2$-D_3 action suggests that reduction of NF-κB DNA binding by 1,25-$(OH)_2$-D_3 requires de novo protein synthesis in MRC-5 cells (Fig. 2).

3.3. Reduction of NF-κB binding by 1,25-$(OH)_2$-D_3 is not restricted to the IL-8 promoter NF-κB binding site

Further EMSA experiments were performed to investigate whether the repression of NF-κB binding occurs on NF-κB binding sites other than that in the IL-8 promoter. Nuclear extracts were incubated with radiolabeled oligonucleotides representing the NF-κB binding site of the human immunodeficiency virus long terminal repeat (HIV-LTR), the NF-κB binding site of the p50 promoter at nucleotides −11 to −2 relative to the transcription start [24] and the NF-κB binding site of the IL-6 promoter. In all cases the binding of NF-κB was reduced in nuclear extracts from cells preincubated with 1,25-$(OH)_2$-D_3 before treatment with IL-1β, showing that this

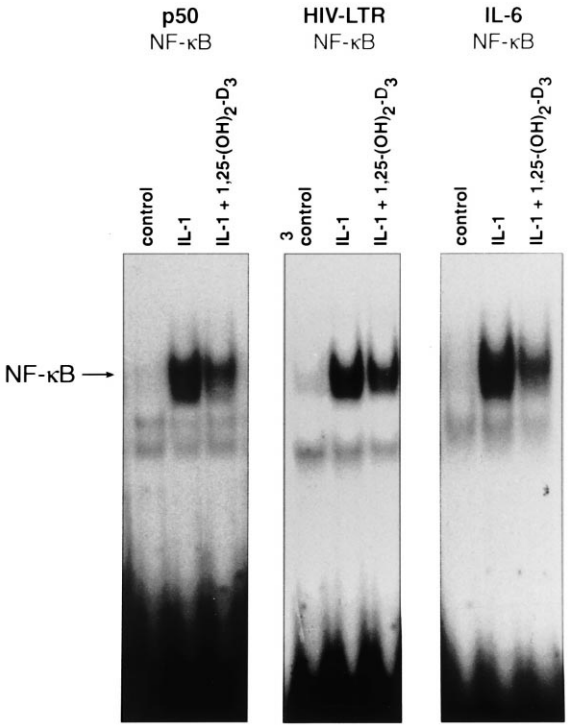

Fig. 3. Reduction of NF-κB binding by 1,25-$(OH)_2$-D_3 is not restricted to the IL-8 promoter NF-κB binding site. 2×10^6 cells were treated with 1,25-$(OH)_2$-D_3 (20 nM) for 23 h preceding a 1 h stimulation with IL-1β (50 ng/ml) or with IL-1β alone. For EMSAs, nuclear extracts were incubated with radiolabeled oligonucleotides representing one NF-κB binding site of the p50 promoter (p50 NF-κB), the NF-κB binding site of the HIV long terminal repeat (HIV-LTR NF-κB) or the NF-κB binding site of the IL-6 promoter (IL-6 NF-κB).

phenomenon is not restricted to the IL-8 NF-κB binding site (Fig. 3).

3.4. *Cytokine-induced IL-8 and IL-6 mRNA levels are down-regulated by 1,25-(OH)$_2$-D$_3$ and dexamethasone in MRC-5 fibroblasts*

Northern blot experiments demonstrated that stimulation of MRC-5 cells with IL-1β (50 ng/ml) for 4 h resulted in induction of IL-8 mRNA and, at lower levels, IL-6 mRNA. When cells were preincubated for 20 h with 1,25-$(OH)_2$-D_3 or dexamethasone, or a combination of both before 4 h stimulation with IL-1β, a reduction of IL-1β-induced IL-8 mRNA levels was observed (Fig. 4a). IL-1β-induced IL-6 mRNA levels were moderately reduced by 1,25-$(OH)_2$-D_3, while dexamethasone had a more pronounced effect (Fig. 4b). TNF-α induced much lower levels of IL-8 mRNA in MRC-5 cells than IL-1β, while induction of IL-6 mRNA by TNF-α was almost undetectable. This observation is consistent with the findings of Ng et al. who reported that IL-6 and IL-8 mRNA levels are differentially regulated by TNF-α and IL-1β in the human fibroblast cell lines MRC-9 and FS-4. The higher induction of IL-6 and IL-8 mRNA by IL-1β is due to enhanced transcription rates and mRNA stability induced by IL-1β compared to TNF-α [25]. However, treatment of MRC-5 cells with 1,25-$(OH)_2$-D_3 (20 nM), dexamethasone (1 μM) or both caused a reduction of TNF-α-induced IL-8 mRNA levels in all cases (Fig. 4a).

3.5. *Translocation of p50 and p65 is not inhibited by 1,25-(OH)$_2$-D$_3$ in MRC-5 cells*

Immunofluorescence analysis was performed using MRC-5 cells treated with either 1,25-$(OH)_2$-D_3 (20 nM), dexamethasone (1 μM) or a combination of both for 23 h preceding a 1 h stimulation with IL-1β (50 ng/ml). Staining of cells for p50 or p65 demonstrated translocation of both NF-κB subunits to the nucleus induced by 1 h treatment with IL-1β, but neither 1,25-$(OH)_2$-D_3 nor dexamethasone significantly altered this translocation (Fig. 5).

4. Discussion

Chronic inflammatory diseases, such as psoriasis or rheumatoid arthritis, are characterized by dysregulation of expression of chemotactic peptides, triggering leukocyte infiltration into the inflamed tissue [26–28]. Clinically such chronic disorders can be treated with antiinflammatory compounds, which act via inhibition of proinflammatory and chemotactic cytokines, such as IL-8.

The sequences −94 to −71 relative to the transcription start of the IL-8 gene have been shown to be essential for both activation and repression of the IL-8 promoter by various agents [29–36], mainly involving the transcription factor NF-

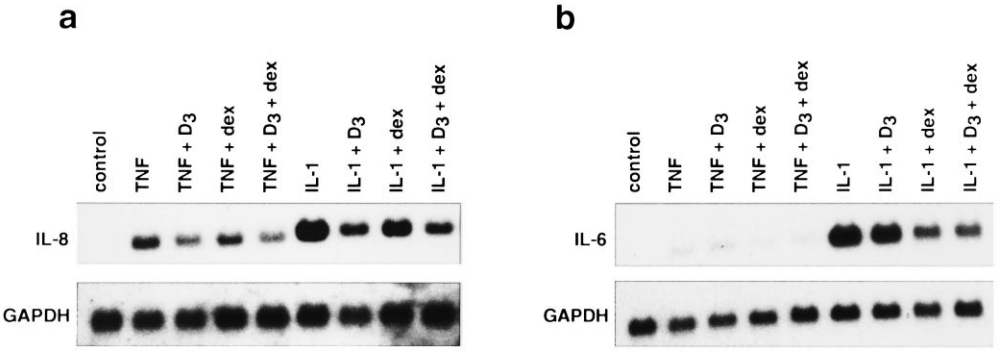

Fig. 4. Cytokine-induced IL-8 and IL-6 mRNA levels are downregulated by 1,25-$(OH)_2$-D_3 or dexamethasone in MRC-5 cells. 5×10^5 cells were seeded into each well of a six-well plate and treated with 1,25-$(OH)_2$-D_3 (D_3, 20 nM), dexamethasone (dex, 1 μM), 1,25-$(OH)_2$-D_3 and dexamethasone for 20 h before stimulation with either TNF-α (200 U/ml) or IL-1β (50 ng/ml) for 4 h. Total RNA was isolated and about 10 μg of each total RNA was loaded onto an agarose-formaldehyde gel. After transfer, the blots were hybridized with a ^{32}P-labeled human IL-8 cDNA probe (a) or a ^{32}P-labeled human IL-6 cDNA probe (b) at 65°C overnight. Blots were exposed to X-ray films at −70°C for 4 h (IL-8) and 24 h (IL-6). After stripping the same blots were rehybridized with a ^{32}P-labeled rat GAPDH cDNA probe.

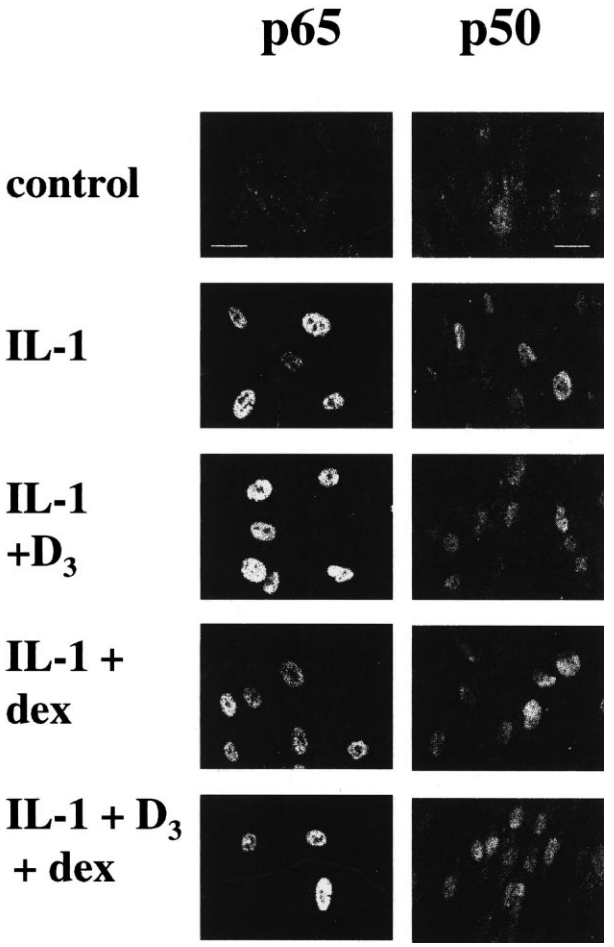

Fig. 5. IL-1β-induced translocation of p50 and p65 is not inhibited by 1,25-$(OH)_2$-D_3 or dexamethasone in MRC-5 cells. Cells were treated with 1,25-$(OH)_2$-D_3 (D_3, 20 nM), dexamethasone (dex, 1 μM) or a combination of both for 23 h before stimulation with IL-1β (50 ng/ml) for 1 h. Immunofluorescence was performed as described in Section 2. Bar, 25 μm.

κB. A classical repressor of NF-κB-driven genes, such as IL-8, is the glucocorticoid dexamethasone, which mediates its effects via the glucocorticoid receptor (GR). One mechanism for glucocorticoid inhibition of inflammatory cytokine expression is GR-NF-κB interaction, which has been reported to occur at different levels, either directly with the p65 subunit of NF-κB [37–41] or via upregulation of its inhibitor IκBα, which then retains NF-κB in the cytoplasm [42–45]. In contrast to glucocorticoids, the effects of 1,25-$(OH)_2$-D_3 on NF-κB activity have not been well studied, and the mechanism is less clear. Yu et al. have shown that 1,25-$(OH)_2$-D_3 can reduce levels of p50 and its precursor p105 in human lymphocytes and they observed reduced binding of PMA-induced nuclear factors to the NF-κB binding site of the IL-6 promoter [20]. We have previously shown that 1,25-$(OH)_2$-D_3 downregulates TNF-α-induced IL-8 promoter activity in the human melanoma cell line G-361, accompanied by reduced binding of NF-κB [21]. It was further demonstrated that 1,25-$(OH)_2$-D_3 can transcriptionally downregulate expression of the p40 subunit of IL-12, and requires the presence of VDR and RXR [22]. To establish whether 1,25-$(OH)_2$-D_3 could be a potentially useful antiinflammatory agent and to evaluate its role in NF-κB regulation, we investigated its effect in fibroblasts, which have been shown to contribute to disease processes by production of inflammatory cytokines [46]. We demonstrate here that 1,25-$(OH)_2$-D_3 can partially inhibit the activity of NF-κB in MRC-5 normal human fibroblasts. While translocation of its subunits p50 and p65 is not affected by 1,25-$(OH)_2$-D_3, its major activity is inhibition of the DNA binding of NF-κB, which we observed on a variety of NF-κB binding sites and which appears not to be mediated directly by VDR. Similar results were also obtained by D'Ambrosio et al. who showed partial inhibition of NF-κB DNA binding by 1,25-$(OH)_2$-D_3 in THP-1 human monocytic cells [22].

Although activation of NF-κB plays a fundamental role in the transcription of IL-8 and IL-6, mRNA levels of these cytokines are only moderately downregulated by 1,25-$(OH)_2$-D_3. We conclude that reduction of NF-κB binding by 1,25-$(OH)_2$-D_3 is not sufficient to strongly repress expression of these genes in MRC-5 cells and that additional events, such as interference with the transactivation potential of NF-κB or interaction with other nuclear factors, are required for optimal repression of IL-8 or IL-6 transcription. However, enhancement of mRNA stability by IL-1 may counteract regulatory events which occur at the level of transcription.

The mechanism by which 1,25-$(OH)_2$-D_3 reduces NF-κB binding does not appear to involve direct protein-protein interaction of NF-κB and VDR of the type reported for the GR, but requires protein synthesis, as cycloheximide abrogated the negative effect of 1,25-$(OH)_2$-D_3. Also, recombinant human VDR and RXRα themselves were unable to bind the NF-κB binding site or to compete NF-κB from its site in the IL-8 promoter (data not shown), observations consistent with the findings of D'Ambrosio et al. [22]. It may therefore be possible that 1,25-$(OH)_2$-D_3 can upregulate expression of another factor(s), which then contribute to the inhibition of NF-κB binding.

The ability of 1,25-$(OH)_2$-D_3 to inhibit NF-κB activity in cell types involved in inflammation clearly contributes to the notion of 1,25-$(OH)_2$-D_3 as an antiinflammatory principle, and supports clinical use of 1,25-$(OH)_2$-D_3 either alone or in combination with 'classical' inhibitors of inflammation. Future studies will therefore focus on this activity of 1,25-$(OH)_2$-D_3 and on synthetic analogs, which may have more pronounced activity than the parent hormone.

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References

[1] Bouillon, R., Okamura, W.H. and Norman, A.W. (1995) Endocr. Rev. 16, 200–257.
[2] Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. and Evans, R.M. (1995) Cell 83, 835–839.
[3] Demay, M.B., Gerardi, J.M., DeLuca, H.F. and Kronenberg, H.M. (1990) Proc. Natl. Acad. Sci. USA 87, 369–373.
[4] Zierold, C., Darwish, H.M. and DeLuca, H.F. (1994) Proc. Natl. Acad. Sci. USA 91, 900–902.
[5] Noda, M., Vogel, R.L., Craig, A.M., Prahl, J., DeLuca, H.F. and Denhardt, D.T. (1990) Proc. Natl. Acad. Sci. USA 87, 9995–9999.
[6] Cheskis, B. and Freedman, L.P. (1994) Mol. Cell. Biol. 14, 3329–3338.

[7] Tsoukas, C.D., Provvedini, D.M. and Manolagas, S.C. (1984) Science 224, 1438–1439.
[8] Schüle, R., Umesono, K., Mangelsdorf, D.J., Bolado, J., Pike, J.W. and Evans, R.M. (1990) Cell 61, 497–504.
[9] Alroy, I., Towers, T.L. and Freedman, L.P. (1995) Mol. Cell. Biol. 15, 5789–5799.
[10] Takeuchi, A., Reddy, G.S., Kobayashi, T., Okano, T., Park, J. and Sharma, S. (1998) J. Immunol. 160, 209–218.
[11] Siebenlist, U., Franzoso, G. and Brown, K. (1994) Annu. Rev. Cell Biol. 10, 405–455.
[12] Thanos, D. and Maniatis, T. (1995) Cell 80, 529–532.
[13] Collins, T., Read, M.A., Neish, A.S., Whitley, M.Z., Thanos, D. and Maniatis, T. (1995) FASEB J. 9, 899–909.
[14] Baeuerle, P.A. and Baltimore, D. (1996) Cell 87, 13–20.
[15] Mercurio, F., Zhu, H., Murray, B.W., Shevchenko, A., Bennett, B.L., Li, J., Young, D.B., Barbosa, M., Mann, M., Manning, A. and Rao, A. (1997) Science 278, 860–866.
[16] Woronicz, J.D., Gao, X., Cao, Z., Rothe, M. and Goeddel, D.V. (1997) Science 278, 866–869.
[17] Régnier, C.H., Song, H.Y., Gao, X., Goeddel, D.V., Cao, Z. and Rothe, M. (1997) Cell 90, 373–383.
[18] DiDonato, J.A., Hayakawa, M., Rothwarf, D.M., Zandi, E. and Karin, M. (1997) Nature 388, 548–554.
[19] Zandi, E., Rothwarf, D.M., Delhase, M., Hayakawa, M. and Karin, M. (1997) Cell 91, 243–252.
[20] Yu, X.-P., Bellido, T. and Manolagas, S.C. (1995) Proc. Natl. Acad. Sci. USA 92, 10990–10994.
[21] Harant, H., Andrew, P.J., Reddy, G.S., Foglar, E. and Lindley, I.J.D. (1997) Eur. J. Biochem. 250, 63–71.
[22] D'Ambrosio, D., Cippitelli, M., Cocciolo, M.G., Mazzeo, D., Di Lucia, P., Lang, R., Sinigaglia, F. and Panina-Bordignon, P. (1998) J. Clin. Invest. 101, 252–262.
[23] Harant, H., de Martin, R., Andrew, P.J., Foglar, E., Dittrich, C. and Lindley, I.J.D. (1996) J. Biol. Chem. 271, 26954–26961.
[24] Cogswell, P.C., Scheinman, R.I. and Baldwin Jr., A.S. (1993) J. Immunol. 150, 2794–2804.
[25] Ng, S.B., Tan, N.H. and Guy, G. (1994) J. Biol. Chem. 269, 19021–19027.
[26] Gillitzer, R., Berger, R., Nielke, V., Muller, C., Wolff, K. and Stingl, G. (1991) J. Invest. Dermatol. 97, 73–78.
[27] Brennan, F.M., Zachariae, C.O.C., Chantry, D., Larsen, C.G., Turner, M., Maini, R.N., Matsushima, K. and Feldmann, M. (1990) Eur. J. Immunol. 20, 2141–2144.
[28] Peichl, P., Ceska, M., Effenberger, F., Haberbauer, G., Broell, H. and Lindley, I.J.D. (1991) Scand. J. Immunol. 34, 333–339.
[29] Mukaida, N., Okamoto, S., Ishikawa, Y. and Matsushima, K. (1994) J. Leukocyte Biol. 56, 554–558.
[30] Mukaida, N., Mahe, Y. and Matsushima, K. (1990) J. Biol. Chem. 265, 21128–21133.
[31] Yasumoto, K., Okamoto, S., Mukaida, N., Murakami, S., Mai, M. and Matsushima, K. (1992) J. Biol. Chem. 267, 22506–22511.
[32] Oliveira, I.C., Mukaida, N., Matsushima, K. and VilCek, J. (1994) Mol. Cell. Biol. 14, 5300–53008.
[33] Mukaida, N., Morita, M., Ishikawa, Y., Rice, N., Okamoto, S., Kasahara, T. and Matsushima, K. (1994) J. Biol. Chem. 269, 13289–13295.
[34] Kunsch, C. and Rosen, C.A. (1993) Mol. Cell. Biol. 13, 6137–6146.
[35] Stein, B. and Baldwin Jr., A.S. (1993) Mol. Cell. Biol. 13, 7191–7198.
[36] Kunsch, C., Lang, R.K., Rosen, C.A. and Schannon, M.F. (1994) J. Immunol. 153, 153–164.
[37] Ray, A. and Prefontaine, K.E. (1994) Proc. Natl. Acad. Sci. USA 91, 752–756.
[38] Scheinman, R.I., Gualberto, A., Jewell, C.M., Cidlowski, J.A. and Baldwin Jr., A.S. (1995) Mol. Cell. Biol. 15, 943–953.
[39] Caldenhoven, E., Liden, J., Wissink, S., Van de Stolpe, A., Raaijmakers, J., Koenderman, L., Okret, S., Gustafsson, J.A. and Van der Saag, P.T. (1995) Mol. Endocrinol. 9, 401–412.
[40] De Bosscher, K., Schmitz, M.L., vanden Berghe, W., Plaisance, S., Fiers, W. and Haegeman, G. (1997) Proc. Natl. Acad. Sci. USA 94, 13504–13509.
[41] Wissink, S., van Heerde, E.C., Schmitz, M.L., Kalkhoven, E., van der Burg, B., Baeuerle, P.A. and van der Saag, P.T. (1997) J. Biol. Chem. 272, 22278–22284.
[42] Auphan, N., DiDonato, J.A., Rosette, C., Helmberg, A. and Karin, M. (1995) Science 270, 286–290.
[43] Scheinman, R.I., Cogswell, P.C., Lofquist, A.K. and Baldwin Jr., A.S. (1995) Science 270, 283–286.
[44] Brostjan, C., Anrather, J., Csizmadia, V., Stroka, D., Soares, M., Bach, F.H. and Winkler, H. (1996) J. Biol. Chem. 271, 19612–19616.
[45] Heck, S., Bender, K., Kullmann, M., Göttlicher, M., Herrlich, P. and Cato, A.C.B. (1997) EMBO J. 16, 4698–4707.
[46] Schröder, J.-M., Sticherling, M., Henneicke, H.H., Preissner, W.C. and Christophers, E. (1990) J. Immunol. 144, 2223–2232.