The nuclear factor-κB (NF-κB) protein RelB plays a unique role in dendritic cell (DC) function and, as such, is an important regulator of antigen presentation and immune regulation. In this study, inhibition of RelB expression in DCs exposed to an analog of the active form of vitamin D₃ (1α,25-dihydroxyvitamin D₃ (1α,25-(OH)₂D₃)) was observed and shown to be mediated by the vitamin D receptor (VDR). Potential vitamin D response elements were identified within promoter regions of human and mouse reLB genes. In gel shift experiments, these motifs specifically bound VDR-retinoid X receptor-α complexes. Reporter assays confirmed that transcriptional activity of human and mouse reLB promoters was inhibited by 1α,25-(OH)₂D₃ agonists in a DC-derived cell line. The inhibition was abolished by mutagenesis of the putative vitamin D response elements and was enhanced by overexpression of VDR. Mutagenesis of NF-κB response elements within the reLB promoter did not affect the magnitude of 1α,25-(OH)₂D₃ analog-mediated inhibition, ruling out an indirect effect on NF-κB signaling. Glucocorticoid caused additional inhibition of reLB promoter activity when combined with the 1α,25-(OH)₂D₃ analog. This effect was dependent on the integrity of the NF-κB response elements, suggesting separate regulatory mechanisms for the two steroid pathways on this promoter. We conclude that reLB is a direct target for 1α,25-(OH)₂D₃-mediated negative transcriptional regulation via binding of VDR-retinoid X receptor-α to discrete DNA motifs. This mechanism has important implications for the inhibitory effect of 1α,25-(OH)₂D₃ on DC maturation and for the potential immunotherapeutic use of 1α,25-(OH)₂D₃ analogs alone or combined with other agents.

Dendritic cells (DCs) occupy a unique role in initiating immune responses as a result of their ability to mingle with and potently activate naïve T-cells (1, 2). A burgeoning literature also demonstrates an important function for DCs in maintaining peripheral immune tolerance (3). The degree to which DC function can be polarized to induce immune sensitization or tolerance is highlighted by advances toward the therapeutic use of DCs to both boost (for neoplasia and vaccination) and inhibit (for transplantation and autoimmunity) antigen-specific cellular immunity (1, 2, 4, 5). This functional plasticity is linked with a collection of phenotypic changes (termed maturation) that convert the DC from a cell with modest antigen-presenting capacity to one with high surface levels of peptide-major histocompatibility complex complexes and co-stimulatory ligands (1, 2). Triggering of the DC maturation program is induced by engagement of surface receptors for microbial products, pro-inflammatory cytokines, and co-receptors expressed by activated T-cells (1, 2). Maturational stimuli are channeled through intracellular signaling cascades, the targeting of which has been identified as a key strategy in modulating DC phenotype for the purpose of immunotherapy (6).

Prominent among the signals that regulate DC maturation is the nuclear factor-κB (NF-κB) pathway (1, 2, 6, 7). Rel/NF-κB proteins are a family of transcription factors that serve as pivotal regulators of immune, inflammatory, and acute-phase responses (8–10). Rel/NF-κB proteins are involved in the modulation of immune responses in vivo (13). Inhibition of RelB nuclear translocation in DCs may be critical in regulating the differentiation and maturation of DCs. RelB-deficient mice lack mature myeloid DCs (11, 12), and DCs in which RelB expression is inhibited retain an immature phenotype and are associated with induction of immune tolerance in vivo (13). Inhibition of RelB nuclear translocation in DCs has also been observed following the use of tolerogenic immunosuppressive regimens in experimental models of allograft transplantation (14).

We have recently reported that the active form of the steroid reLB; 1α,25-(OH)₂D₃, 1α,25-dihydroxyvitamin D₃; VDR, vitamin D receptor; VDRE, vitamin D response element; RXRα, retinoid X receptor-α; IL, interleukin; NF-κB-RE, nuclear factor-κB response element; BMDCs, bone marrow-derived dendritic cells; PBS, phosphate-buffered saline.
hormone 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3) and its analogs, which are known to potently inhibit DC maturation (15), selectively inhibit mRNA and protein expression of RelB in bone marrow-derived DCs (15, 16). The inhibition of RelB in DCs is further attenuated by addition of glucocorticoid, and DCs generated in the combined presence of 1α,25-(OH)2D3 and glucocorticoid agonists exhibit a highly immature phenotype (16). The inhibitory effects of 1α,25(OH)2D3 of its analogs are predominantly mediated by the vitamin D receptor (VDR), which then acts as a transcriptional regulator by binding to vitamin D response elements (VDREs) within the promoters of responsive genes, most commonly as a heterodimer with retinoid X receptor-α (RXRα) (17, 18). Negative regulation by 1α,25-(OH)2D3 of immune-related gene products such as interleukin (IL)-2, interferon-γ, and IL-12 p40 has been documented, but it has not been possible to clearly identify VDREs in the promoters of these genes (19–21). In this report, we present evidence that 1α,25(OH)2D3-mediated inhibition of RelB in DCs is a VDR-dependent process that operates through bona fide VDREs within the promoter regions of both human and mouse RelB genes and that may be augmented by concurrent interference with separate NF-κB response elements (NF-κB-REs) in the relB promoter.

MATERIALS AND METHODS

Experimental Animals, Reagents, and Antibodies—VDR knockout and littermate wild-type VDR mice (provided by Dr. Marie DeMay, Massachusetts General Hospital, Boston, MA) (22) were bred and maintained in a specific pathogen-free facility. Crystalline preparations of 1α,25-(OH)2D3 and of the vitamin D3 analog 1α,25(OH)2D3-16-ene-23-yn-26,27-hexafluoro-19-nor-D3 (subsequently referred to as D3 analog) were provided by Dr. Milan Uskokovic (Hoffman-La Roche) and stored under nitrogen at −80 °C as stock solutions in absolute alcohol. The antibodies and detection agents used in this study were as follows: anti-mouse RelB polyclonal antibody (Santa Cruz Biotechnology), anti-VDR polyclonal antibody (NeoMarkers Inc., Fremont, CA), Alexa Fluor 488-conjugated goat anti-rabbit IgG (Molecular Probes, Inc., Eugene, OR), and horseradish peroxidase-conjugated protein A (Amersham Biosciences). All oligonucleotides were synthesized by Integrated DNA Technologies (Corvallis, IA).

Cell Culture and Transient Transfection—Murine bone marrow-derived DCs (BMDCs) were prepared as described previously (23). D3 analog and dexamethasone (Sigma) were added on days 2, 4, and 6 of culture to final concentrations of 10−8 and 10−7 M, respectively. Mouse D2SC1 cells (provided by Dr. Sang-Mo Kang, University of California, San Francisco, CA) (24) were cultured in Iscove’s modified Dulbecco’s medium containing L-glutamine, penicillin/streptomycin, and 5% fetal bovine serum. Cells were transiently transfected with luciferase reporter plasmids, the pRL-TK reference luciferase plasmid (Promega, Madison, WI), and expression plasmids using FuGENE 6 reagent (Roche Applied Science) in accordance with the manufacturer’s instructions.

Indirect Immunofluorescence—Day 7 BMDCs from wild-type VDR and VDR knockout mice were seeded on 10-well microscope slides (Erie Scientific Co., Portsmouth, NH), fixed in 3% paraformaldehyde for 15 min on ice, washed three times with phosphate-buffered saline (PBS), permeabilized in 0.2% Triton X-100 in PBS for 10 min, and washed with PBS. After blocking for 1 h in PBS and 5% nonfat dry milk, cells were incubated with anti-mouse RelB polyclonal antibody (1:10) for 1 h at room temperature, followed by three washes with PBS and 5% nonfat dry milk. Finally, cells were incubated with secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit IgG, 4 µg/ml) for 45 min in PBS and 5% nonfat dry milk, followed by three washes. Slides were mounted with Vectashield mounting medium (Vector Laboratories, Inc., Burlingame, CA) containing DAPI and a 1:1 mixture of 1:1000 anti-VDR antibody, washed, incubated with a 1:8000 dilution of horse- radish peroxidase-conjugated protein A, and then visualized using the ECL detection system (Amersham Biosciences).

Luciferase Reporter Assays—Mouse D2SC1 cells were seeded in 6-well plates at 5 × 105 cells/well. Twenty-four hours later, cells were transfected with 1 µg of plasmid-encoded promoter construct and 10 ng of pRL-TK plasmid (encoding Renilla luciferase under the control of the thymidine kinase promoter) as an internal control. In some experiments, the cells were cotransfected with 0.5 µg of mVDR expression construct in pcDNA3.1 and 10 ng of pRL-TK plasmid to quantitate the amount of mVDR coding region. Cells were washed with ice-cold PBS, harvested, resuspended in lysis buffer (50 mM HEPES, pH 7.9, 150 mM NaCl, 1 mM EDTA, 0.1% Nonidet P-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, 10 µg/ml antipain, 10 µg/ml aprotinin, and 10 µg/ml pepstatin), and kept on ice for 15 min. After preclaring, the whole cell lysate protein was quantified using the Bio-Rad protein assay kit. Aliquots of 50 µg were separated on 10% precast Tris-HCl gels (Bio-Rad) and transferred to Immobilon-P membrane (Millipore Corp., Bedford, MA). Membranes were blocked in 5% nonfat dry milk, incubated with a 1:400 dilution of anti-VDR antibody, washed, incubated with a 1:8000 dilution of horse-radish peroxidase-conjugated protein A, and then visualized using the ECL detection system (Amersham Biosciences).

 Luciferase Reporter Assays—Mouse D2SC1 cells were seeded in 6-well plates at 5 × 105 cells/well. Twenty-four hours later, cells were transfected with 1 µg of plasmid-encoded promoter construct and 10 ng of pRL-TK plasmid (encoding Renilla luciferase under the control of the thymidine kinase promoter) as an internal control. In some experiments, the cells were cotransfected with 0.5 µg of mouse VDR expression construct in pcDNA3.1. Ten hours later, the medium was removed and replaced with control medium or with medium containing D3 at final concentrations of between 10−8 and 10−4 M with or without 10−7 M dexamethasone. After an additional 24 h, the cells were harvested and assayed for reporter gene activity and Renilla luciferase activity using the dual-luciferase assay kit (Promega) according to the manufacturer’s instructions. Final results for each sample were recorded as Renilla adjusted relative light units.

Data Analysis—All experiments were carried out a minimum of three times with consistent results. For all reporter assays, duplicate or triplicate samples for each condition were prepared, and final results are expressed as means ± S.D. Statistical differences between individual experimental conditions were determined using two-tailed, unpaired Student’s t test with significance assigned to p < 0.05.
Radiolabeled oligonucleotides containing human motifs A and B were carried out with no addition (CONTROL) or in the presence of D3 analogs (D3) or dexamethasone. Bone marrow cultures were carried out with no addition (CONTROL) or in the presence of D3 analogs or of dexamethasone and were stained for RelB. Cytoplasmic and nuclear staining of DCs was clearly present in control cultures from both animals (left panels). Reduced staining for RelB was evident following D3 analog treatment of cultures from wild-type VDR bone marrow, but not from D3 knockout bone marrow (middle panels). Exposure of either wild-type VDR or D3 knockout bone marrow cultures to dexamethasone resulted in reduced RelB immunofluorescence (right panels).

RESULTS

Inhibited DC Expression of RelB by 1α,25-(OH)2D3 Agonist Is a VDR-dependent Process—To determine whether 1α,25-(OH)2D3-mediated inhibition of RelB in DCs is dependent on the expression of the VDR, BMDCs were generated from wild-type VDR and VDR-deficient mice in the absence or presence of an optimized concentration (23) of D3. BMDCs were stained strongly for RelB (Fig. 1). Cultures treated with the glucocorticoid dexamethasone were also examined. Untreated day 7 BMDCs stained strongly for RelB, while cultures treated with 1α,25-(OH)2D3 were stained less strongly. A clear reduction in RelB immunofluorescence was observed in wild-type BMDCs by both D3 analog and dexamethasone in wide-type VDR BMDCs, but not by dexamethasone in VDR-deficient BMDCs. The results are consistent with a VDR-mediated inhibitory action of D3 analog on RelB expression.

Human and Mouse relB Promoter Regions Contain Putative VDREs That Bind to the VDR-RXRα Complex—Genomic DNA sequences were used to identify potential VDREs that bind to the VDR-RXRα complex. Genomic DNA sequences were sequenced to the start codons of the human and mouse relB genes were examined for potential VDREs. The canonical DR3 VDRE hexamer ((A/G)G(T/G)TCA) as a benchmark (18), motifs consisting of two hexameric repeats conforming to a N(G/C)N(T/A)(G/C)(T/A) sequence and separated by three nucleotides were sought. Two such sequences were identified in the human relB promoter region (designated as human relB motif A (~799 to ~785) and human relB motif B (~443 to ~429)), and one was identified in the mouse promoter region (designated as the mouse relB motif (~602 to ~588)). The positions and sequences of these motifs are illustrated in Fig. 2A. The sequences of the mutated motifs that were generated for use as controls in subsequent experiments are shown in Fig. 2B. The abilities of these three putative VDREs to complex with VDR and RXRα were tested in gel shift experiments (Fig. 3). Radiolabeled oligonucleotides containing human motifs A and B were found to complex with VDR and RXRα together, but not with either protein alone (Fig. 3A). The binding was competed in a dose-dependent fashion by non-radiolabeled oligonucleotides containing the same sequence or the sequence of a positive regulatory VDRE from the mouse osteopontin promoter (26), but not by oligonucleotides containing an AP-1-binding site. Radiolabeled oligonucleotides in which the putative VDREs were mutated demonstrated an absent or markedly reduced ability to complex with VDR and RXRα. Comparable results were obtained with oligonucleotides incorporating the mouse wild-type and mutant relB motifs (Fig. 3B). The relative affinity of the mouse motif for VDR and RXRα was compared with that of the human motifs and with the osteopontin VDRE in a competitive gel shift assay (Fig. 3C). The ability of human motifs A and B to compete with the radiolabeled mouse motif was less than that of the mouse motif itself, whereas the osteopontin VDRE competed more potently than any of the relB sequences. At a 10-fold excess of non-radiolabeled oligonucleotide, the mouse relB motif was associated with a 50% reduction in the density of the shifted band compared with 16, 30, and 93% for human motifs A and B and the osteopontin motif, respectively (Fig. 3C). We concluded that the identified sequences from the human and mouse relB genes, including the putative VDREs, were ligated into a luciferase-encoding plasmid and employed in reporter assays using the murine DC-derived cell line D2SC1 (24). Detectable low-level expression of VDRE by this cell line was confirmed at the mRNA and protein levels (data not shown). Constructs were also generated in which the putative VDR motifs were mutated to sequences shown by gel shift to have little affinity for VDR-RXRα (Figs. 2B and 3). For the human construct, motifs A and B were mutated singly and together. Promoter activity for this panel of reporter constructs were measured in D2SC1 cells in the absence and presence of D3 analog and are expressed as the percent reduction associated with D3 analog treatment (Fig. 4, two similar experiments shown). Both human and mouse wild-type promoter activities were significantly inhibited by 1α,25-(OH)2D3 agonist. In >10 separate experiments, D3 analog treatment was associated with a consistent significant reduction in both human and mouse wild-type promoter activities that varied between 30 and 80%. In contrast, the human double mutant VDRE and mouse mutant VDRE promoters were minimally inhibited (0–10% in multiple experiments). The human single mutants were inhibited by D3 analog to a lesser degree than the human wild-type promoter, although the difference did not consistently reach statistical significance.

The influence of VDR expression levels on inhibition of human and mouse relB promoter activities by D3 analog was next examined using the same reporter assay protocol with cotransfection of a plasmid encoding mouse VDR or an empty expression vector (Fig. 5). The concentration of D3 analog was titrated from 10 μM to 10–8 M, and the results were compared with untreated D2SC1 cells in the absence or presence of VDRE overexpression. For both human and mouse relB promoters, the absolute promoter activity was significantly lower, and the percent reduction compared with that in untreated cells was greater at all concentrations of D3 analog for the VDRE-overexpressing cells. For example, at the suboptimal concentration of 10–12 M D3 analog, the percent reduction in promoter activity for cells overexpressing VDR was 40% for the human relB promoter and 45% for the mouse relB promoter compared with 25 and 9%, respectively, for cells not overexpressing VDR. At the optimal concentration of 10–13 M, the equivalent results...
were 86 and 71% versus 68 and 43% (Fig. 5). In multiple reporter assay experiments, closely comparable results were obtained when 1α,25-(OH)2D3 was substituted for D3 analog (data not shown).

The results clearly support the contention that the VDR-RXRα-binding motifs identified in the human and mouse relB promoters represent negative regulatory VDREs and are necessary for 1α,25-(OH)2D3-mediated inhibition of RelB expression in DCs. Furthermore, the magnitude of 1α,25-(OH)2D3-mediated inhibition of relB gene transcription in DCs is influenced by the expression level of VDR.

**Inhibition of the relB Promoter by 1α,25-(OH)2D3 Agonist Is Independent of NF-κB-REs, but the Additive Effects of Glucocorticoid Are Mediated through NF-κB-REs—Transcriptional expression of the human relB gene is positively regulated by two NF-κB-REs (25). As 1α,25-(OH)2D3 has been reported to interfere with NF-κB signaling (21, 27), the effect of eliminating the NF-κB-REs on relB promoter activity in D3 analog-treated D2SC1 cells was determined. Cells were cotransfected with VDR along with the human wild-type relB reporter construct or with a construct in which the two NF-κB-REs were inactivated by mutagenesis (see Fig. 2B for the sequences of wild-type and mutant NF-κB-REs) and were exposed to graded concentrations of D3 analog. As shown in Fig. 6, reporter activity from the human mutant NF-κB-RE promoter was consistently lower than that from the wild-type promoter, but the degree of inhibition by each concentration compared with that in untreated cells was very similar for both constructs. At D3 analog concentrations of 10−12, 10−10, and 10−8 M, the wild-type promoter activity was inhibited by 40, 86, and 89%, respectively, whereas equivalent degrees of inhibition for the mutant NF-κB-RE promoter were 58, 92, and 89%. The ability of a glucocorticoid agonist (dexamethasone) to additively inhibit relB promoter activity in combination with D3 analog was then tested using the human wild-type and mutant NF-κB-RE constructs (Fig. 7A). In contrast, no additional dexamethasone-associated inhibition of the mutant NF-κB-RE promoter occurred. The equivalent NF-κB-REs from the mouse relB promoter were also identified and mutated (see Fig. 2B). As shown in Fig. 7B, the effect of dexamethasone in combination with D3 analog on mouse wild-type and mutant NF-κB-RE relB promoters was closely comparable to the results obtained with the human construct. In the experiments shown, the addition of 10−7 M dexamethasone to 10−10 M D3 analog resulted in an increase in the degree of inhibition of promoter activity from 28 to 55% for the human wild-type relB promoter and from 31 to 52% for the mouse relB promoter. For the human and mouse mutant NF-κB promoters, the degree of inhibition for D3 analog alone was 33 and 26%, respectively, compared with 25 and 25% for D3 analog and dexamethasone. Comparable results were obtained in multiple repeat experiments. We concluded that 1α,25-(OH)2D3-mediated inhibition of relB promoter activity in DCs operates independently of NF-κB-REs, but is capable of additively inhibiting relB gene transcription when combined with an antagonist of NF-κB signaling such as glucocorticoid.

**DISCUSSION**

The results of this study demonstrate a direct negative regulatory effect of 1α,25-(OH)2D3 on the promoter region of the gene encoding RelB, a pivotal NF-κB component in the regulation of DC differentiation and maturation (11–14). The potential binding motifs for VDR-RXRα that were identified in both mouse and human promoters proved to have specific affinity for recombinant VDR-RXRα in gel shift experiments. Further-
more, using a panel of luciferase reporter constructs, it was possible to demonstrate that 1α,25-(OH)₂D₃- and D₃ analogs-mediated negative regulation of relB promoter activity occurs in a DC-derived cell line and is dependent upon the presence of the VDR-RXRα-binding motifs. Although two VDREs were identified in the human promoter and only one in the mouse promoter, the inhibitory effects of 1α,25-(OH)₂D₃ agonists on the two promoters were closely comparable, an observation that may be explained by the relatively higher affinity of the mouse VDRE for VDR alone, recombinant RXR alone, or combined VDR and RXR (lanes 1–4); subjected to PAGE, and imaged. The portions of the gels containing shifted bands are shown. In separate incubations, a series of non-radiolabeled competitor oligonucleotides (Comp.) were added at increasing ratios (10:1 (20 pmol), 50:1 (100 pmol), and 100:1 (200 pmol) for each) in the presence of VDR-RXRα (lanes 5–13). Competing oligonucleotides were the individual relB motifs themselves (Self; lanes 5–7), a canonical VDRE from the mouse osteopontin gene promoter (lanes 8–10), and an AP-1 response element (AP-1 RE; lanes 11–13). Oligonucleotides containing mutated sequence at the putative VDREs (Mutant) were also tested (lanes 14–17). In C, the radiolabeled mouse relB motif was incubated with VDR and RXRα in the absence of a competing non-radiolabeled oligonucleotide (lane 1) or in the presence of graded amounts of competing oligonucleotides containing the same sequence (lanes 2–4), the putative human relB VDREs (motif A; lanes 5–7) and motif B (Human B; lanes 8–10), and the mouse osteopontin VDRE (lanes 11–13). The proportionate reduction in the density of the major shifted band compared with the control reaction (lane 1) is shown at the bottom of the lanes for each competitive reaction (% RED).

results in the generation of immature DCs that are associated with antigen-specific suppression of secondary T-cell responses when administered to sensitized animals.

The immunomodulatory effects of the vitamin D endocrine system have been studied for >20 years, and in vitro and in vivo studies have identified the DC as a primary target of 1α,25-(OH)₂D₃ or related analogs, with or without additional immunosuppressive agents, was associated with protection against autoimmunity and allograft rejection and with expansion of CD4⁺/CD25⁺ regulatory T-cell populations. These observations were suggested to result from in vivo modulation of DC/T-cell interactions to favor the generation of antigen-specific regulatory T-cell populations, a mechanism that has been evoked by others to explain the tolerance induced by inoculation with or targeting of antigen to immature DCs (3, 34). Although it is clear that additional individual genes may be regulated in cells of the immune system by 1α,25-(OH)₂D₃ agonists (15), our finding of direct transcriptional suppression of a key signaling protein (RelB) represents a discrete VDR-mediated mechanism whereby such agents may promote
“Tolerogenic” antigen presentation. Furthermore, the separate effects of D₃ analog and glucocorticoid on the relB promoter provide a mechanistic basis for the additive or synergistic effects of 1α,25-(OH)₂D₃ agonists on immune-mediated disease (35).

The demonstration that the magnitude of transcriptional repression of relB by 1α,25-(OH)₂D₃ agonists is influenced by the level of VDR expression has important implications for in vivo potency of immunomodulatory D₃ analogs. Human tonsillar DCs (generally a site of ongoing active immune responses) constitutively express VDR (36), whereas lymphocyte-depleted mouse splenocytes (a mixture of macrophage/monocytes and DCs) demonstrate induction of VDR following a retroviral infection (37). Hewison et al. (38) have also demonstrated that VDR expression undergoes regulation during DC differentiation from monocytes. The fact that VDR is an inducible protein within immune cell populations suggests that immunotherapy using D₃ analogs is likely to target the DCs involved in an emerging or established immune injury. With regard to RelB repression, this would imply that newly recruited DCs and DCs undergoing maturation-inducing stimulation may be specifically modified by 1α,25-(OH)₂D₃ and related analogs to retain an immature phenotype. Whether VDR is regulated in DCs by additional endogenous or exogenous factors remains to be determined. It is interesting, however, that Cantorna et al. (39) have identified an interplay between dietary calcium and protection against autoimmunity in 1α,25-(OH)₂D₃-treated animals. Polymorphisms of the VDR gene have also been linked with predisposition to immune-mediated disease (40). Although the mechanisms for these observations are not known at present, it is likely that environmental and genetic factors that influence base-line and inducible VDR expression also affect susceptibility to 1α,25-(OH)₂D₃-mediated immunosuppression.

The possible mechanisms whereby 1α,25-(OH)₂D₃ bound to VDR-RXRα negatively regulates transcription of certain genes
include competitive displacement of positive regulatory transcription factor complexes, recruitment of corepressor proteins, and direct interference with assembly of the transcriptional machinery. Regarding 1α,25-(OH)2D3-mediated inhibition of immune-related genes, Cippitelli and Santoni (20) demonstrated that two VDR-RXRα-binding regions in the interferon-γ promoter are responsible for negative regulation of this gene, with the potential to interfere with both AP-1 recruitment and transcriptional complex assembly. D’Ambrosio et al. (21) characterized the inhibition by 1α,25-(OH)2D3 of IL-12 p40 promoter activity in DCs as being mediated through interference with NF-κB transcriptional activation, but did not detect direct binding of VDR to this promoter region. Alroy et al. (19) and Takeuchi et al. (41) identified a region within the human IL-2 promoter in which a VDR-RXRα-binding domain and an NFATp (nuclear factor of activated T-cells p) domain overlap. Interestingly, the DNA region to which VDR-RXRα bound did not closely conform to any reported VDREs, and the corresponding mouse sequence failed to bind VDR-RXRα. DNA-bound VDR-RXRα was shown to complex with NFATp and to destabilize its association with AP-1 components. Towers and Freedman (42) characterized a variant VDRE half-site in the promoter of the granulocyte/macrophage colony-stimulating factor gene that overlaps with an NFATp/AP-1 site and that mediates transcriptional repression upon binding VDR alone. The VDREs we have identified in the mouse and human relB promoters conform more closely to canonical DR3 VDREs than those described for the IL-2 and granulocyte/macrophage colony-stimulating factor promoters and do not detectably bind VDR alone. We have not, to date, identified a potential overlapping binding site for positive regulatory complexes associated with the relB promoter VDREs, and our results with mutant NF-κB-RE promoter constructs rule out the possibility that binding of VDR-RXRα to the VDREs acts by interfering with the function of these NF-κB-REs. The characterization of nuclear proteins associated with DNA-bound VDR-RXRα complexes in DCs and of the other signaling pathways involved in relB transcription may provide additional insights.

In conclusion, we have shown that the promoter region of the gene encoding the NF-κB family member RelB is a direct target of the vitamin D system in mouse and human via one or more non-classical hexameric repeats that directly bind VDR-RXRα and that mediate negative transcriptional regulation. The unique influence of RelB expression on DC function identifies this novel mechanism as a key element in the immunotherapeutic properties of 1α,25-(OH)2D3 and its analogs.

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Regulation of RelB by Vitamin D

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Direct Transcriptional Regulation of RelB by 1α,25-Dihydroxyvitamin D₃ and Its Analogs: PHYSIOLOGIC AND THERAPEUTIC IMPLICATIONS FOR DENDRITIC CELL FUNCTION

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